

PATHOLOGY

Official Organ for the American Society For Experimental Pathology

**Problems in the Pathologic Diagnosis of
Carcinoma of the Thyroid**

Robert C. Horn Jr.

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Carcinoma of the Thyroid Gland**

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A. M. A.

ARCHIVES of PATHOLOGY

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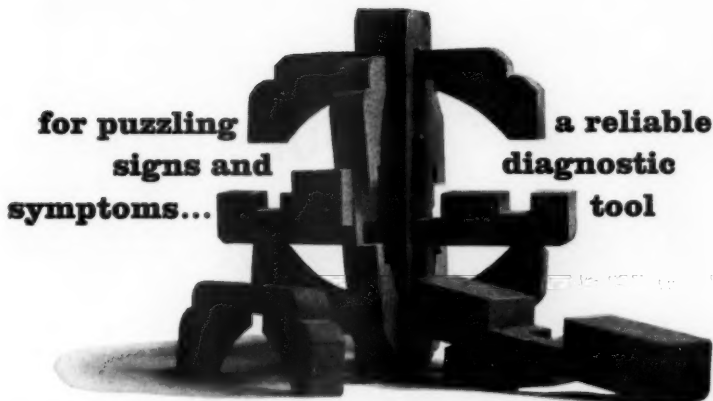
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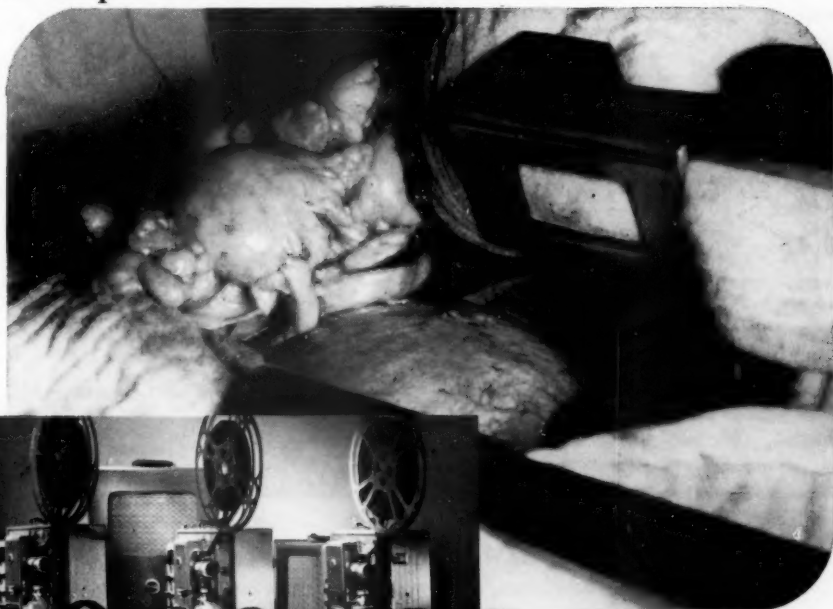
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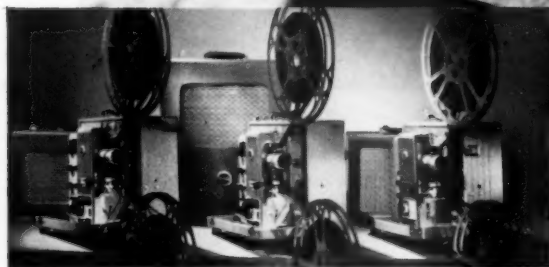
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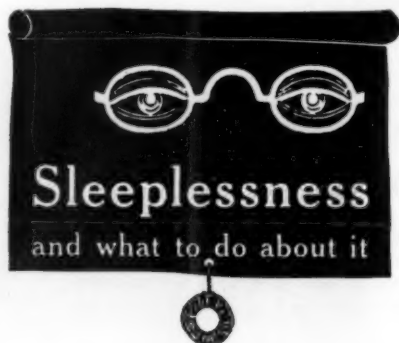
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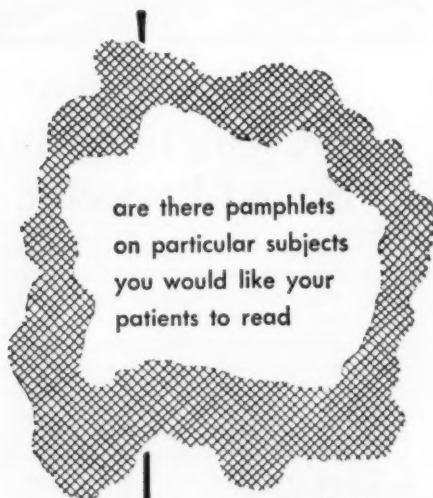


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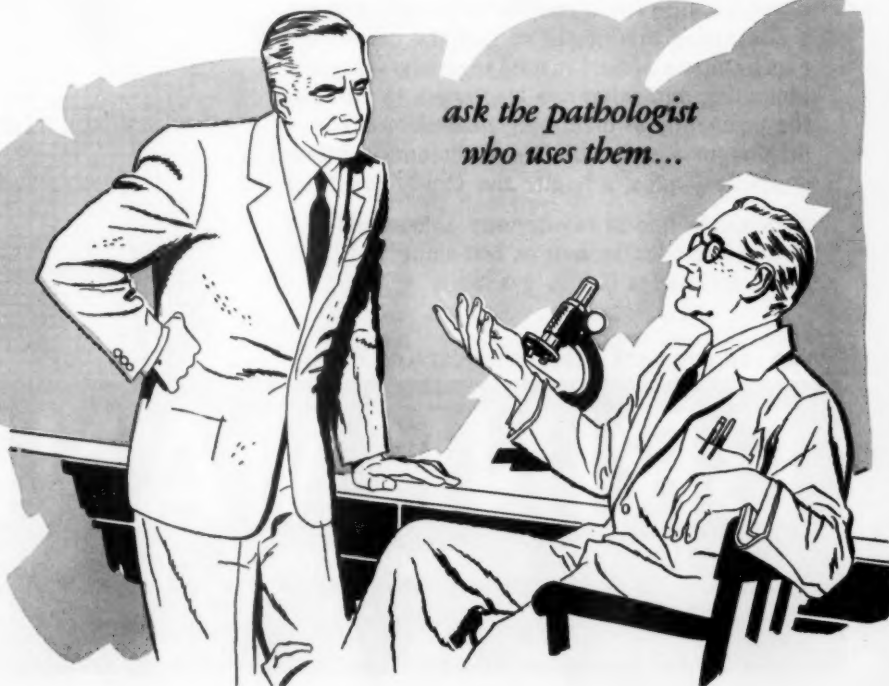


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A.M.A. ARCHIVES OF PATHOLOGY

Problems in the Pathologic Diagnosis of Carcinoma of the Thyroid

ROBERT C. HORN Jr., M.D., Detroit

The difficulty of histologic diagnosis of some diseases of the thyroid gland is a matter of fairly general agreement among pathologists.^{8,10,12,13} In the last decade or so, as the result of many excellent investigations, publication of numerous long-term follow-up studies, and the widespread participation of pathologists in seminars and workshops, many of the problems have been at least partially solved. However, there remain some biologically proved cancers that do not have the familiar histopathologic features of malignancy, and, in addition, an apparently increasing number of benign lesions whose morphologic resemblance to cancer leads the alert pathologist, anxious not to let a malignant tumor go unrecognized, to suspect carcinoma where none exists. The present study was undertaken in an attempt to identify factors responsible for such problems and, if possible, to clarify diagnostic criteria.

Materials and Methods

Sixty-eight cases are included in this study. Fifty-nine of these were seen in consultation, sought in hope of support of an opinion, for help, or because of my known particular interest in

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From the Department of Laboratories, Henry Ford Hospital.

Read before the Section on Pathology and Physiology at the 108th Annual Meeting of the American Medical Association, Atlantic City, June 11, 1959.

the subject, but always with some degree of uncertainty as to diagnosis. Whereas an individual case may present a pathologist with an insoluble immediate problem, the problem may often be resolved by the study of a group of similar cases. I am indebted to 22 colleagues for the opportunity to study these 59 cases. Only nine cases are included from the Henry Ford Hospital material; it was difficult to apply the criterion of selection used above, namely, that the case was enough of a problem to one pathologist to cause him to seek the opinion of another. The Henry Ford Hospital cases are those in which the diagnosis was reversed or remains in doubt; they do not by any means include all the cases that presented diagnostic difficulties. They represent a little less than 1% of the thyroid glands studied in the surgical pathology laboratory during this time period.

The clinical data and pathologic material of all cases have been restudied, and each case has finally been classified in one of six groups, as follows: nodular goiter, 3 cases; adenoma, 10 cases; carcinoma, 22 cases; "thyroiditis," including lymphoid goiter, 12 cases; hyperplasia, 11 cases, and diagnosis uncertain, 10 cases. These groups are not always mutually exclusive; cases having two of the above diagnoses have arbitrarily been placed in the group that would best facilitate discussion.

It is unfortunate that the final classification cannot be corroborated by long-term follow-up study in all cases. Although follow-up information has been obtained in most of the cases included here, the period of follow-up observation in some is relatively short. Nevertheless, study of this material has yielded certain useful information and, in many instances, has afforded confidence in a previously disputed diagnosis, even in the absence of biologic proof.

Results

Age and sex followed very much the usual pattern for studies of thyroid disease. Only 9 of the 65 patients whose sex is known were male. Four of these were finally classified as having carcinoma, and in three cases the diagnosis still remains problematical. This series is perhaps weighted slightly with younger patients, no doubt a reflection of hesitancy in making the diagnosis of carcinoma in this age group. Nine patients (of 64 whose ages are known) were in the first two decades of life, and an additional ten were 30 years of age or less.

Nodular Goiter.—The problem of excluding carcinoma in all three cases classified as nodular goiter was occasioned by the presence of papillary structures within a nodule. In one instance the papillary fronds were attenuated and atrophic; in the other two, thick and fibrotic. In one of the latter the papillary structure was observed within a cyst partially lined by squamous epithelium and showed a considerable inflammatory-cell infiltrate. None of these cases was actually diagnosed as carcinoma.

Adenoma.—Of the 10 cases finally classified as adenoma, 7 were histologically atypical. In five the atypia were found to consist of growth in more or less solid sheets or trabeculae with poor follicle formation and little or no colloid. One was wholly com-

posed of oxyphil or Hürthle cells. Although well circumscribed, it had no capsule. The sixth atypical adenoma was composed of festooned trabeculae of elongated cells oriented perpendicularly, and, as in the preceding case, lacked a capsule (Fig. 1). The last atypical adenoma showed, in part, an alveolar grouping of plump or elongated, pale cells (Fig. 2A) and, elsewhere, a peculiar papillary pattern, where the papillary cores were thick and the covering cells cuboidal and single-layered (Fig. 2B). This lesion also showed striking endothelial proliferation within many of its vessels. In none of these seven lesions was invasion of capsule or vessels found, although it was frequently not possible to examine eight blocks, as Hazard and Kenyon^{5,6} have recommended. Study of these cases supports the authors' contention that the atypical adenoma of the thyroid can be considered benign if capsular or vascular invasion is not found after careful study, although our follow-up is not as complete as theirs. Nevertheless, it is well to remind ourselves, in view of what we know about the frequent very slow evolution of thyroid carcinoma, that some of these atypical adenomas may prove to be malignant if we carry our follow-up studies to multiples of the traditional five-year period. This warning is particularly pertinent with respect to the current series.

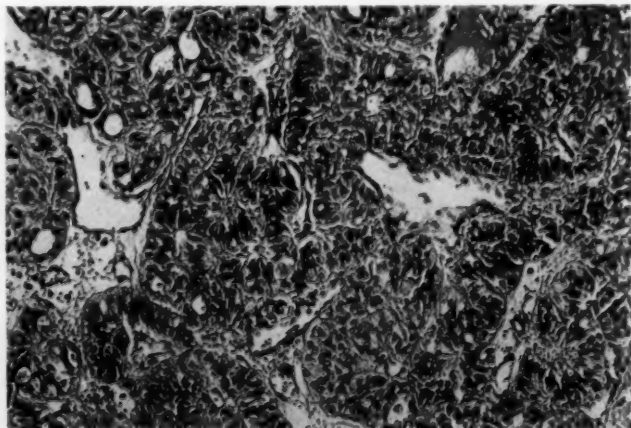


Fig. 1.—Atypical adenoma of thyroid, $\times 150$.

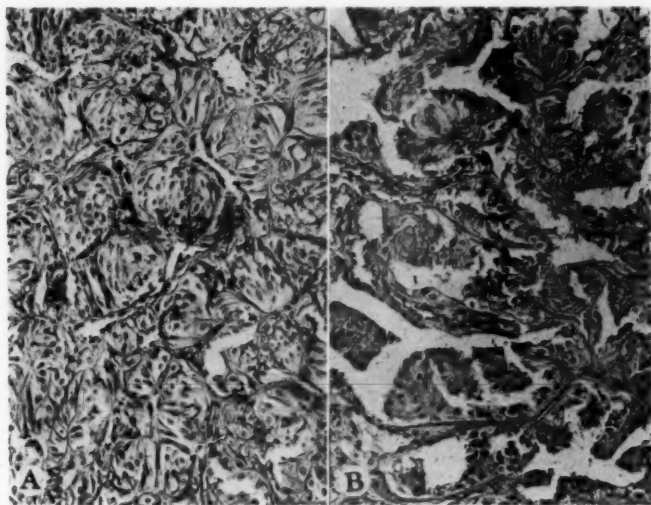


Fig. 2.—Atypical adenoma of thyroid. *A*, alveolar or solid structure; *B*, papillary pattern; reduced to 77% of mag. $\times 150$.

One of the problem cases was a well-differentiated follicular adenoma without invasion but with a papillary structure centrally. The latter was entirely orderly. The ninth adenoma was a small nodule with a thick capsule in which there was an area simulating invasion (Fig. 3A). Because of the completely innocent histologic appearance of the lesion and the presence of inflammatory cells in the area, it was regarded as "pseudoinvasion" produced by reactive fibrosis.

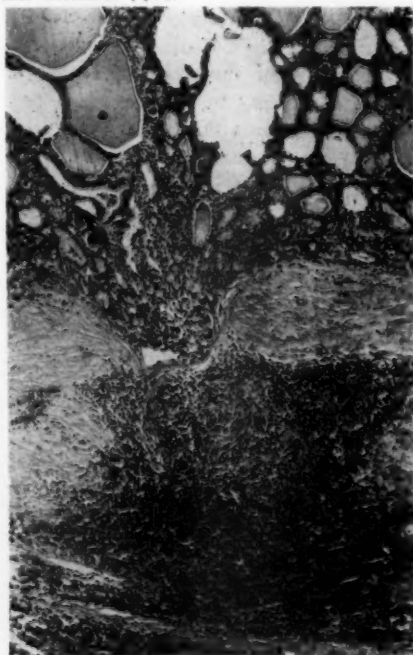
The question of muscle invasion was raised in connection with the remaining adenoma. Actually, the adenoma, encapsulated, was separated from the muscle by essentially normal thyroid follicles intermingled with muscle fibers. Gardiner² has explained this phenomenon as a normal variation, and Meissner¹⁰ has warned that hyperplasia, as well as carcinoma, may produce this appearance of simulated muscle invasion.

Two of the adenomas in this group were originally diagnosed by at least one qualified observer as carcinoma and by three others as possible or probable carcinoma.

Carcinoma.—In 8 of the 22 cases classified as carcinoma, consultation was sought simply for confirmation of the original diagnosis of carcinoma. In two additional

instances multicentric, well-differentiated follicular carcinomas were not recognized, although the patients were known to have thyroid cancer from previous tissue examination (a cervical lymph node in one case; a "solitary nodule" in the other). In one

Fig. 3A.—Adenoma of thyroid. Simulated capsular invasion. $\times 70$.



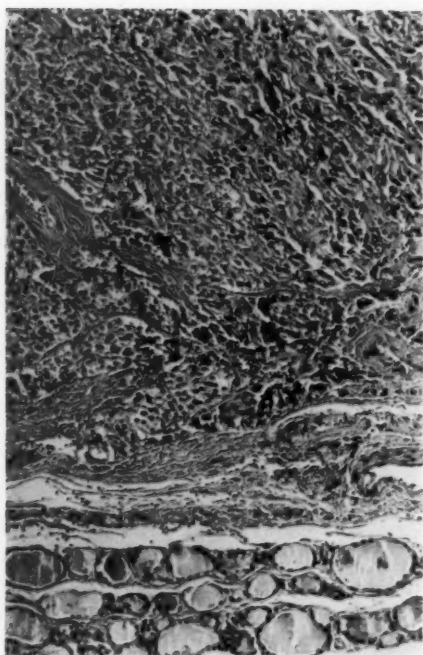


Fig. 3B.—Carcinoma (malignant adenoma type) of thyroid. Capsular invasion. $\times 150$.

instance of a solid tumor with some papillary areas the thyroid origin of the lesion was a matter of disagreement among the original observers. In seven cases of carcinoma the diagnosis was disputed, or there

was a particular problem in differential diagnosis. The alternative was hyperplasia in two cases, adenoma in four, and thyroiditis in one. In one case (Case 30) the tumor was a multicentric, well-differentiated follicular carcinoma. The patient had received propylthiouracil for several months prior to operation, and the question of thiouracil effect was raised. However, both capsule and vessel invasion were demonstrated. In another (Case 38), the problem revolved about the differentiation of carcinoma from a hyperplastic reaction to an area of necrosis in a focal lymphocytic goiter. The lesion had the morphologic character of cancer, and, in addition, there was invasive growth. Of the four cases in which the differential diagnosis included carcinoma and adenoma, two showed capsule invasion (Fig. 3B) and two, blood-vessel invasion (Fig. 4A). One of these four was a well-differentiated follicular tumor composed of Hürthle cells; two others, also composed of Hürthle cells, were more or less solid, with some papillary areas and very little follicle formation (Fig. 4B). The fourth showed the distinctive alveolar pattern which I described and which Hazard and his associates⁴ called medullary or solid (Fig. 5). In one case it was difficult to distin-

Fig. 4.—Carcinoma (malignant adenoma type) of thyroid. A, blood-vessel invasion; reduced to 80% of mag. $\times 50$. B, solid growth of Hürthle cells with occasional suggestion of papillary pattern; reduced to 78% of mag. $\times 150$.



Fig. 5.—Solid, or “medullary,” carcinoma of thyroid. $\times 150$.

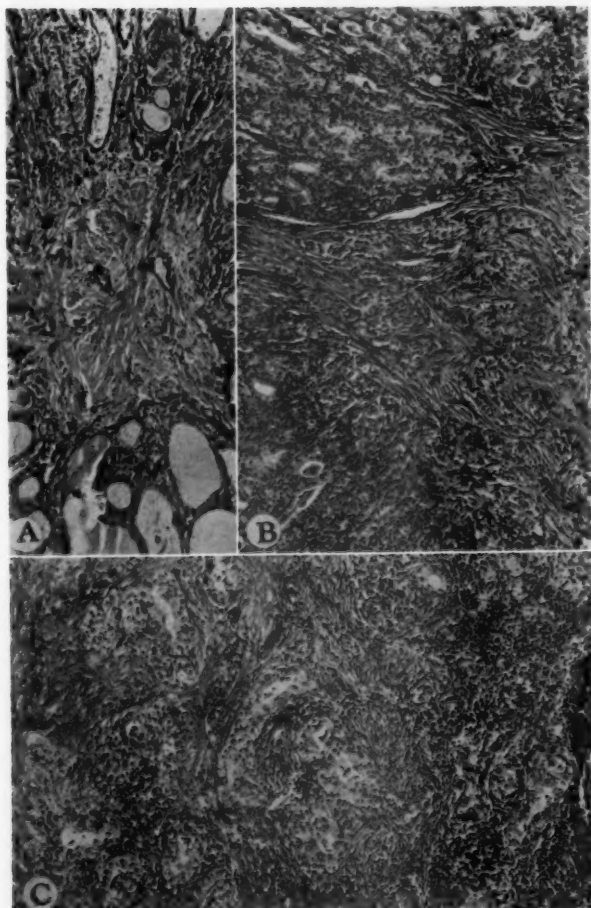
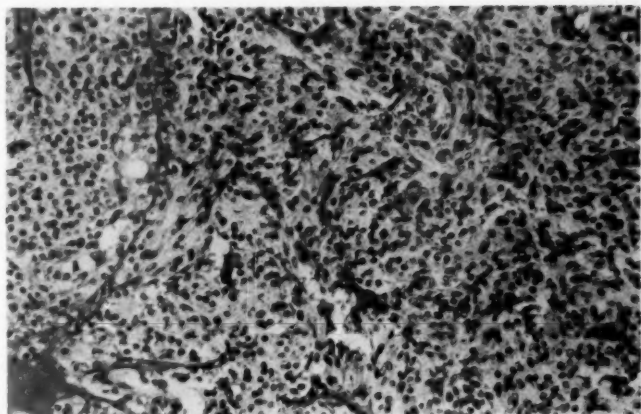


Fig. 6.—Occult carcinomas of the thyroid. *A* and *B* were encountered in diffuse hyperplastic goiters; *C*, in a diffuse lymphocytic goiter. Reduced to 92% of mag. $\times 90$.

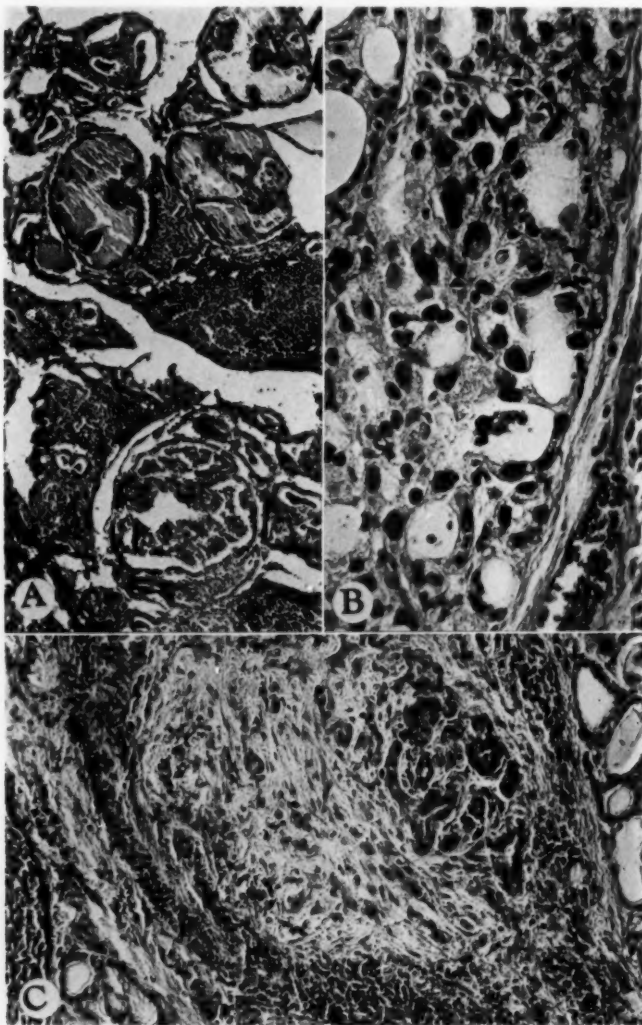
guish between invasive, well-differentiated carcinoma and the simulated invasion of thyroiditis.

In four cases, tiny, sclerosing carcinomas raised the question of differentiation from scars in otherwise diffuse goiters. However, survey of the group reveals a sequence from almost insignificant epithelial elements to the picture of a definite, although still small, carcinoma (Fig. 6). The study of Woolner and his associates¹⁴ suggests that this particular distinction is es-

entially academic. Those occult carcinomas that were incidental findings in their series were not of clinical significance.

A total of 8 carcinomas in this group of 22 thyroids were occult. Two were papillary, showed little sclerosis, and were not associated with hyperplasia, although one gland was diffusely enlarged. The remaining six were follicular and sclerotic. In five cases the glands were otherwise diffusely hyperplastic, and four of these were toxic. One multicentric, occult carcinoma occurred

Fig. 7.—Chronic thyroiditis: three different cases showing (A) hyperplasia, $\times 115$; (B) nuclear pleomorphism and atypical character, $\times 375$; (C) simulated invasive growth, $\times 150$.



in a diffuse lymphocytic goiter with marked oxyphilia. The association of occult carcinomas with diffuse hyperplasia has been noted before; it has been suggested that they are discerned more readily in diffuse than in nodular glands.³

Of the frank carcinomas, seven were follicular and two solid, one of the latter also showing some papillary structure. Only five have been classed as mixed papillary and follicular. This no doubt reflects relative confidence in the diagnosis of papillary carcinoma, as opposed to the well-differentiated tumor composed of colloid-containing follicles.

Of these 22 carcinomas, 5 were originally diagnosed as benign lesions.

"*Thyroiditis*."—This group, together with the hyperplasias, includes the most interesting and important cases in this study. For the purpose of this discussion, we have included under "thyroiditis" both truly inflammatory lesions, usually focal and non-specific, and lymphocytic goiters, also usually focal, a total of 12 cases. Striking papillary hyperplasia (Fig. 7A), marked pleomorphism with bizarre nuclei (Fig. 7B), and an appearance simulating invasion (7C) were each primarily responsible for the diagnostic problem in three cases respectively. In addition, two cases with great

nuclear pleomorphism also raised the question of invasive growth. It appears that a papillary pattern can be safely considered benign in the face of the other characteristic features of thyroiditis; the diffuseness of apparent invasion is usually strongly suggestive of fibrosis and trapping of epithelial elements rather than true invasion, and the bizarre features so often seen in Hürthle cells actually occur more frequently in benign than in malignant lesions of the thyroid, excluding, of course, real anaplasia. In two of the above cases, the lymphoid infiltrate was so dense that lymphoma also entered the differential problem. One case showed squamous metaplasia. Although the occurrence of metaplasia at times raises the question of carcinoma, it has been demonstrated not infrequently in benign conditions.^{1,9,10} The questioned nodule in the 12th case in this group was said to be separate from the thyroid gland proper, although immediately over it and in the midline. The morphology was that of a benign lesion.

Of these 12 tumors, 4 were actually diagnosed as carcinoma and 1 as lymphoma.

Hyperplasia.—In this group are included 8 instances of diffuse hyperplasia, 1 of toxic nodular goiter, 1 of exhaustion atrophy, and an 11th case of hyperplasia and

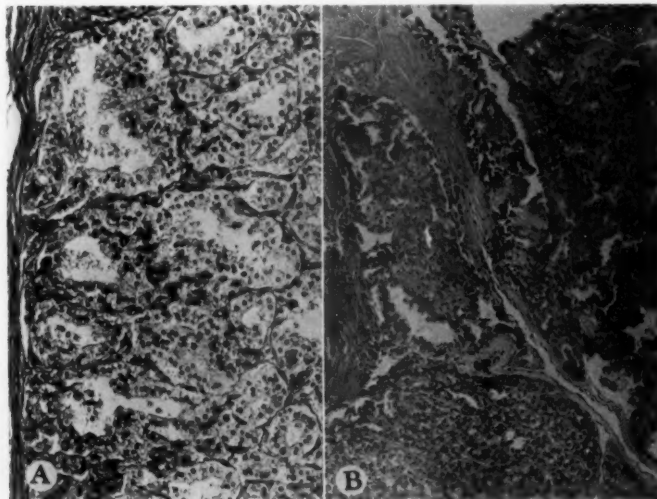


Fig. 8.—A, markedly hyperplastic thyroid showing essentially clear cells; $\times 150$. B, follicular and papillary carcinoma in same gland; reduced to 77% of mag. $\times 115$.

carcinoma, originally considered entirely carcinoma (Fig. 8). In 7 of the first 10 cases, the diagnosis of carcinoma was made initially, and in the other 3 it was seriously considered. The difficulty of differentiating between carcinoma and hyperplasia has been commented on by several authorities.^{8,10,13} In two cases the extreme hyperplasia alone produced confusion with cancer, but in the other nine there were additional factors: atypical nodules, four cases; atypical cytologic pattern, three cases; both

the preceding, one case, and, finally, in one case, the fact that the history of thiocyanate therapy for hypertension was not available at the time of the initial pathologic examination.

Seven of the patients in this group had severe Graves' disease. Three of these were on propylthiouracil treatment for two to six months, but the preoperative preparation of two was completed with iodine. One of these was also treated with cortisone for rheumatoid arthritis for two years. It is

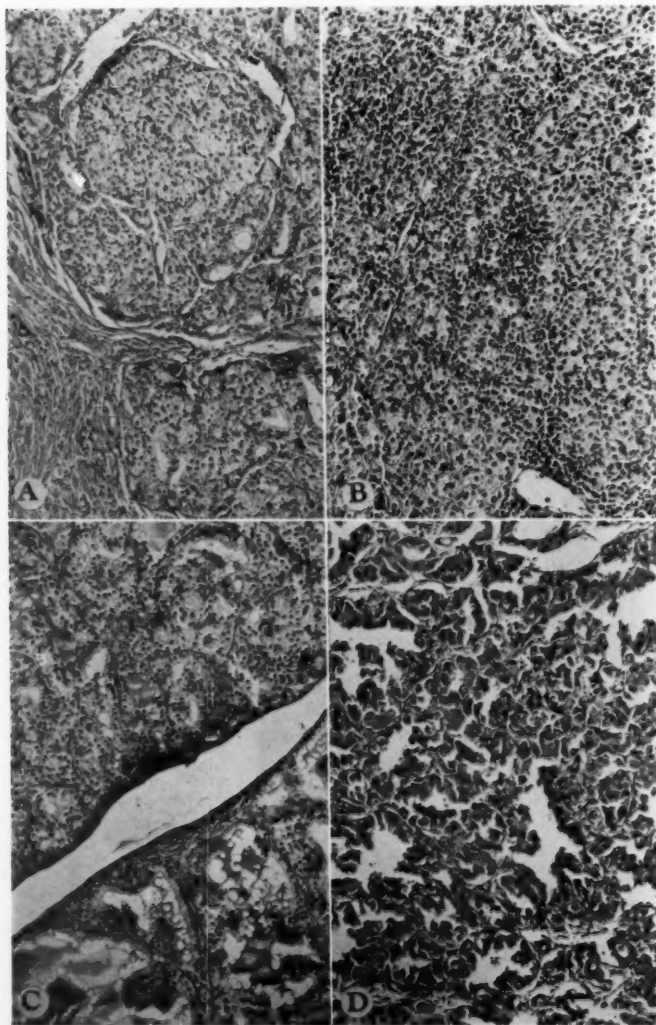


Fig. 9.—Diffuse hyperplastic goiter. *A* and *B* show an almost solid pattern; *C*, a more familiar picture, including both small follicles and large ones with papillary infoldings, and *D*, a striking papillary appearance. Note fibrosis in *A* and diffuse lymphocytic infiltrate in *B*. *A* and *C* are from the same case; reduced to 80% of mag. $\times 115$. *B* and *D* represent two other cases; reduced to 80% of mag. $\times 150$.

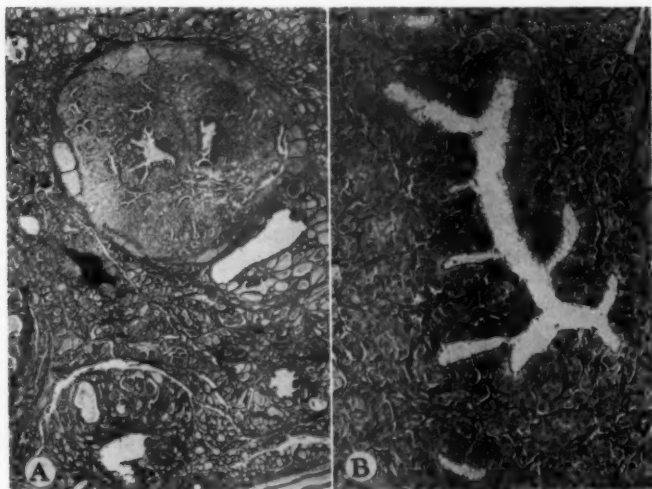


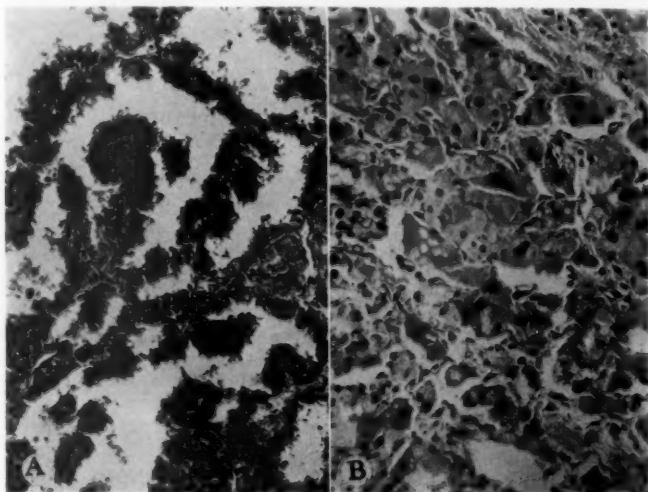
Fig. 10.—Atypical solid nodules with papillary centers that characterized many extremely hyperplastic goiters. Both *A* and *B* reduced to 77% of mag. $\times 13$ and $\times 150$, respectively.

believed that the third patient failed to take her iodine. One patient was prepared for operation with methimazole (Tapazole) and iodine, and one with iodine alone. These goiters tended to be almost solid, with poor follicle formation and little or no colloid (Fig. 9), or to have extensive such solid areas mingled with the more familiar picture of hyperplasia. The cells were large and frequently had clear or very pale cytoplasm. Oxyphilia and cytologic atypia were also common features. In addition, there were distinctive unencapsulated nodules

having a papillary structure quite different from the usual hyperplasia (Fig. 10).

In the two remaining cases of Graves' disease the patients had been toxic for long periods of time. One, whose toxicity was recurrent over a 10-year period and who had had both surgical and x-ray therapy, had a nodular gland in which some nodules were filled with dense colloid and others had a papillary structure without any colloid (Fig. 11*A*). The other had extremely severe thyrotoxicosis for six years, which did not respond to surgical, x-ray, or anti-

Fig. 11.—*A*, papillary hyperplastic nodule in toxic nodular goiter; reduced to 77% of mag. $\times 150$. *B*, exhaustion atrophy of thyroid; reduced to 77% of mag. $\times 250$.



thyroid drug treatment. Her thyroid showed the picture of exhaustion atrophy, with great cellular atypia (Fig. 11*B*).

Of the remaining four patients with hyperplasia, one, as noted, received thiocyanates for hypertension (Fig. 12*A*), and one had been taking 5 grains (0.3 gm.) of thyroid daily for five years. The latter is the patient who had hyperplasia and carcinoma (Fig. 8). The third patient had been treated surgically for thyrotoxicosis 10 years before and for a recurrent non-toxic nodule 5 years before. In the light of this history and the nodular character of the present goiter, it seems probable that the atypical pattern (Fig. 12*B*) is that of

compensatory hyperplasia rather than of carcinoma. Although the nodule in question had no real capsule, true invasive growth was not demonstrated. The fourth case was that of a 20-year-old man whose gland was extremely hyperplastic and papillary, and without colloid (Fig. 12*C*); the patient was not toxic. For some two years before operation the patient had been taking "shots and oral medications" for asthma; it seems probable that this gland belongs to the group of iodide goiters recently reported by Paris and his colleagues.^{11*}

*It has since been learned that the patient's medications included a prescription that contained potassium iodide. The dosage and period of time for which it was taken are unknown.

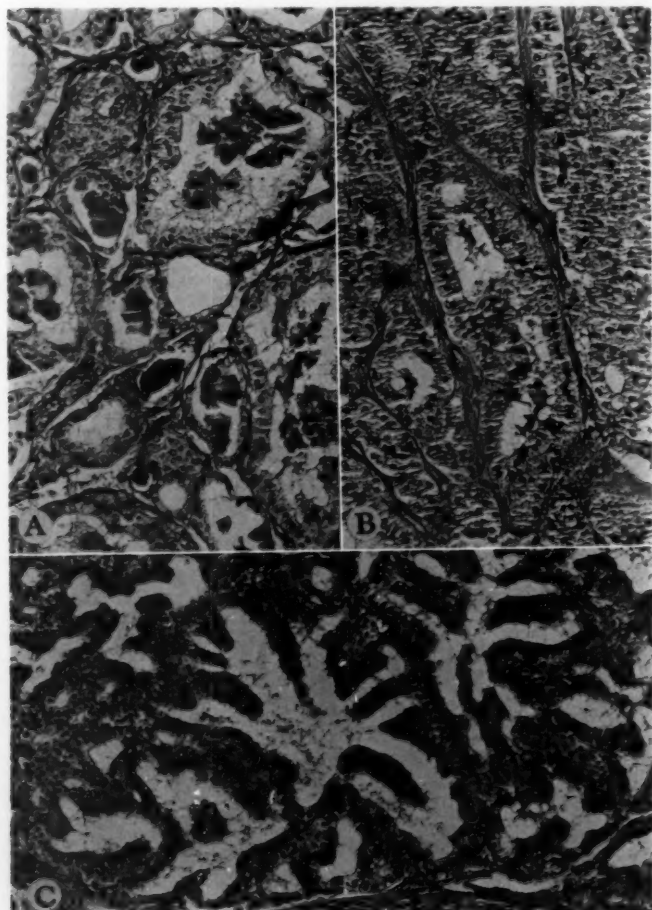


Fig. 12.—*A*, thiocyanate goiter. *B*, compensatory hyperplasia. *C*, diffuse hyperplasia, possibly due to iodides; reduced to 85% of mag. $\times 150$.

Uncertain Diagnostic Cases.—Of the 68 cases in this study, 58 were classified either as carcinoma or as one of a variety of benign lesions with reasonable, although perhaps not complete, assurance. There remain 10 problematic cases, 4 of which were initially called carcinoma and 2 possible carcinoma. One is now considered as probably malignant. This tumor was large, encapsulated, and composed in large part of adult-appearing, colloid-containing follicles. Capsule or vessel invasion was not found, but portions of the lesion showed a distinctive, festooned pattern with duplication of lumina, quite like a pattern known to be associated with malignant metastasizing cancer.

Six of the uncertain cases are now considered to be, in all probability, benign. In four, the lesions have the morphologic appearance of benign adenomas and in one of a nodular goiter with heavy lymphoid infiltrate and fibrosis. However, in all, the occurrence of well-preserved epithelial elements in dense fibrous tissue makes it impossible to rule out invasion with complete confidence. The remaining probably benign lesion was an atypical adenoma in which there was no well-defined capsule, although real capsule or vessel invasion was not demonstrated. However, we were unable to examine multiple blocks. Perhaps, as Park and Lees¹² suggest, for some thyroid lesions we need two standards of "accuracy" of diagnosis—one for practical and one for scientific purposes.

We have been unable to place three cases in any category, even tentatively. One of these is an atypical adenoma in which capsular invasion cannot be excluded and in which there was a recurrent nerve paralysis. The latter cleared partially after operation. The second is a large multinodular goiter with extensive degenerative change in a patient who was mildly toxic. Additional findings were those of a granulomatous thyroiditis plus areas of well-differentiated small follicles suggestive of neoplasm and either growing invasively or simulating invasion because of fibrosis. The last was a

young girl operated upon for a histologically innocent-appearing nodular goiter, who, three years later, had multiple implants of equally innocent-appearing thyroid follicles in the soft tissues of the neck.

Summary

Sixty-eight cases of thyroid gland disease treated surgically that presented difficult or insoluble problems in pathologic diagnosis are reviewed, both pathologically and clinically. The opportunity to study these cases as a group has permitted classification of 58 cases with reasonable assurance that the diagnosis is correct. Among the remaining 10, it is believed that a "probable" diagnosis can be made in 7, 6 of the 7 now being considered probably benign.

Atypical patterns gave rise to diagnostic difficulties among the adenomas and an excellent degree of differentiation among the carcinomas. In both groups the evaluation of invasion, either of vessels or of capsule, was frequently a problem. The latter was also a problem in differentiating thyroiditis from carcinoma and was frequently complicated by a marked degree of cytologic atypia. Hyperplasia of an extreme degree was difficult to differentiate from carcinoma, particularly when accompanied by cytologic atypia as well as nodules of an atypical pattern. Throughout all groups, papillary structures, both hyperplastic and regressive, raised the question of carcinoma. Finally, the importance of a knowledge of the clinical history and gross pathologic description is once again emphasized, particularly in recognizing extremes of hyperplasia.

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REFERENCES

1. Bullock, W. K.; Hummer, G. J., and Kahler, J. E.: Squamous Metaplasia of the Thyroid Gland, *Cancer* 5:966-974, 1952.
2. Gardiner, W. R.: Unusual Relationships Between Thyroid Gland and Skeletal Muscle in Infants: A Review of the Literature and 4 Case Reports, *Cancer* 9:681-691, 1956.

3. Hazard, J. B.; Crile, G., Jr., and Dempsey, W. S.: Nonencapsulated Sclerosing Tumors of the Thyroid, *J. Clin. Endocrinol.* 9:1216-1231, 1949.
4. Hazard, J. B.; Hawk, W. A., and Crile, G., Jr.: Medullary (Solid) Carcinoma of the Thyroid: A Clinicopathologic Entity, *J. Clin. Endocrinol.* 19:152-161, 1959.
5. Hazard, J. B., and Kenyon, R.: Atypical Adenoma of the Thyroid, *A.M.A. Arch. Path.* 58:554-563, 1954.
6. Hazard, J. B., and Kenyon, R.: Encapsulated Angioinvasive Carcinoma (Angioinvasive Adenoma) of the Thyroid Gland, *Am. J. Clin. Path.* 24:755-766, 1954.
7. Horn, R. C., Jr.: Carcinoma of the Thyroid: Description of a Distinctive Morphological Variant and Report of 7 Cases, *Cancer* 4:697-707, 1951.
8. Klinck, G. H.: Thyroid Hyperplasia in Young Children, *J.A.M.A.* 158:1347-1348, 1955.
9. Klinck, G. H., and Menk, F.: Squamous Cells in the Human Thyroid, *Mil. Surgeon* 109: 406-414, 1951.
10. Meissner, W. A.: Seminar on Tumors of the Neck, American Society of Clinical Pathologists, 1956.
11. Paris, J.; McConahey, W. M.; Owen, C. A.; Woolner, L. B., and Bahn, R. C.: Iodide Goiter, paper read before the American Goiter Association, April 30, 1959, Chicago.
12. Park, W. W., and Lees, J. C.: The Histology of Cancer of the Thyroid, *Cancer* 8:320-335, 1955.
13. Warren, S.: Invasion of Blood Vessels in Thyroid Cancer, Editorial, *Am. J. Clin. Path.* 26: 64-65, 1956.
14. Woolner, L. B.; Beahrs, O. H.; Black, B. M., and Keating, F. R., Jr.: Occult Papillary Carcinoma of the Thyroid Gland, paper read before the American Goiter Association, May 2, 1959, Chicago.

Microlithiasis (Calcospherites) and Carcinoma of the Thyroid Gland

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Introduction

The histologic differences between malignant and benign neoplasms of the thyroid gland are sometimes so slight that the presence of well-differentiated metastases in regional lymph nodes is the only evidence of the carcinomatous nature of the neoplasm.

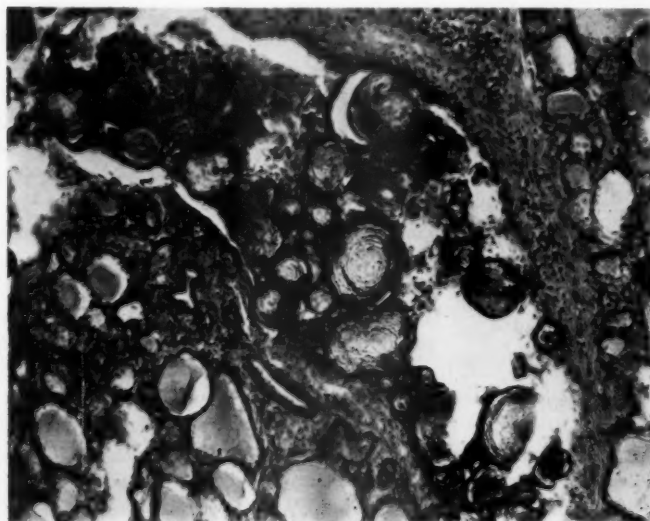
The histopathologic diagnosis of malignant change in some thyroid tumors frequently is based on individual interpretations and experiences. Many of the cytologic criteria of cancer, namely, epithelial distortion, pleomorphism, and the presence of mitotic figures, are observed also in non-cancerous lesions of the thyroid gland. Park and Lees¹ pointed out that even invasion of the stroma cannot be relied upon as an ab-

solute criterion of malignancy. Angioinvasion, pericapsular extension, and local infiltration, although of some diagnostic significance, may also be due to artifactual distortion and/or production.

In addition to the various histologic features referred to, some authors²⁻⁶ consider the presence of calcospherites (psammoma bodies) to be of assistance in differentiating benign from malignant thyroid neoplasms. This report presents evidence to support this concept and represents an extension of a previous survey.²

Methods and Materials

A search for calcospherites was made on 819 thyroid glands removed surgically. The average number of sections examined per case was 10, with a range of 1 to 30. All material was fixed in formalin, and the sections were stained with hematoxylin and eosin.



Numerous calcospherites, showing well-marked lamellated character. These bodies were in an area adjacent to a well-differentiated follicular carcinoma (not shown in photomicrograph). Hematoxylin and eosin; reduced to about 80% of mag. $\times 275$.

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From the Departments of Pathology (Drs. Batsakis and Nishiyama) and Surgery (Dr. Rich) the University of Michigan Medical School.

TABLE 1.—*Calcospherites in Non-Neoplastic and Neoplastic Thyroid Glands*

	Number of Calco- spherite.	Percentage
Non-neoplastic thyroid glands	612	10
Neoplastic thyroid glands	207	84
Total	819	94

Results

Calcospherites (Figure), blue-staining, round, usually laminated bodies, 25μ - 75μ in diameter were found in 84 of 207 malignant epithelial neoplasms of the thyroid gland and in 10 of 612 nonmalignant thyroid glands. The high ratio of carcinoma in this series is more apparent than real, since after an adequate control series of benign glands (612), our investigation was primarily an analysis of thyroid glands in which there was carcinoma.

The malignant neoplasms were classified according to predominant cell pattern. If the papillary and follicular components were approximately equal in the tumor, the carcinoma was classified as "mixed."

The preponderance of calcospherites occurring in papillary neoplasms is readily apparent in Table 2. As noted in our previous study,² the high percentage of calcospherites in the mixed type of carcinoma is due to the bodies occurring in the papillary portion of the neoplasm.

Sex, age, or previous operative procedures had no bearing on the presence of calcospherites. The bodies were present in 10 of 612 non-cancer-bearing glands, an incidence of 1.6%. Here, they were considerably fewer in number than in carcinomas, often only 1 in 8 to 10 sections examined. It is of interest that in the benign lesions in which they appeared, there was some evidence of hyperplasia, either focal, as in treated hyperthyroidism, or a diffuse hyperplasia.

Comment

Pathologists, realizing the difficulties inherent in pure cytologic evidence for thyroid

TABLE 2.—*Calcospherites in Malignant Neoplasms of the Thyroid Gland*

Type of Malignant Neoplasm	Total No. of Malignant Thyroid Neoplasms	Neoplasms with Calcospherites	Percentage with Calcospherites
Papillary	86	43	50.0
Follicular	84	23	27.4
Mixed	17	15	82.4
Undifferentiated (anaplastic)	19	3	15.9
Sarcoma	1	0	0.0
Total	207	84	

carcinoma, have placed reliance on finding capsular violation,⁷ vascular invasion,^{8,9} local aggressiveness, and the presence of obvious metastases to adjacent lymph nodes; each of these criteria is subject to artifactual distortion or production. Even the definition of metastasis has been difficult to establish, since only recently has the "lateral aberrant thyroid" been recognized as a metastasis and not a developmental error.¹⁰

Stout¹¹ indicated the difficulty in assessing capsular invasion in areas of scarring and hemorrhage, while Park and Lees¹ considered stromal invasion to have little value as a criterion of malignant change in the thyroid gland. Warren¹² cautioned against the mere presence of thyroid cells or follicles in the lumen of a vessel being regarded as blood-vessel invasion. At times it may be necessary to resort to special staining techniques to emphasize elastic tissue or other features of the vascular walls and thereby establish an exact, or at least truer, relation between tumor cells and vessels. Vascular invasion may occur in "adenomas" or lesions whose architecture alone would arouse the investigator's doubts as to their benign nature. Even under these circumstances, Lahey, Hare, and Warren¹³ found it to be present in only 3% of such cases. Since well-differentiated carcinomas of the thyroid gland tend to grow slowly, and the correlation between evidences of histologic and "biologic" malignant change may be minimal, vascular invasion is an important diagnostic finding, even though only 10% of

the patients¹³ whose glands showed evidence of vascular invasion by carcinoma had clinical evidence of malignant disease.

Intravascular extension is also subject to variation according to the type of neoplasm; i.e., Graham⁸ did not see invasion of blood vessels in "papilliferous adenocarcinomata" in his original series.

The psammoma body, or "calcospherite," has only recently been stressed as an adjunct to the above criteria or aids in the diagnosis of thyroid carcinoma. Its presence has not been ignored before, but little significance, except in passing, has been placed on its presence. Ewing,¹⁴ discussing papillary neoplasms, noted that "calcific granules may appear in the stroma or alveoli." Anderson¹⁵ mentioned the occasional occurrence of psammoma bodies as one of the distinguishing features of thyroid carcinoma, especially in the papillary type. Warren and Meissner¹⁶ stated: "Small granules of calcification, like grains of sand, are common, but more extensive calcification is infrequent," and, in the legend for Figure 60 in their article: "Formation of calcified bodies resembling psammoma bodies is a common occurrence in the centers of papilliferous processes."

Klinck^{3,17} was the first to emphasize the occurrence of calcospherites and stated that when such bodies were found, a meticulous search should be made for a primary carcinoma of the thyroid gland. Crile and Fisher⁴ noted that the bodies were valuable in the diagnosis of frozen sections of the thyroid gland when the question of papillary carcinoma arose. They specifically utilized the presence of psammoma bodies in their Case 2, in which thyroiditis and papillary carcinoma both were present. Winship and Chase¹⁸ stated that microliths were helpful in diagnosing thyroid cancer and considered that vascular invasion should not be the sole basis for the diagnosis of carcinoma. Underwood and associates⁵ concluded that the presence of psammoma bodies in a papillary lesion of the thyroid gland was an indication that the lesion was malignant. They stated that when the bodies

were numerous, they could be demonstrated radiographically.

In our previous survey² of these bodies in a series of surgically removed thyroid glands, 40.7% of the carcinomas contained calcospherites, and only 2.4% of benign glands possessed similar bodies. The addition of the present series statistically strengthens the diagnostic significance of the presence of calcospherites.

The possibility that other papilliferous epithelial neoplasms might also show numerous calcospherites was investigated. We examined 100 consecutive renal-cell carcinomas from the surgical files of the University of Michigan Department of Pathology; psammoma bodies were found in 4 of 100 cases (4%). Abrams and Tiziani,¹⁹ in a study of ovarian cystadenomas and carcinomas, found a 16% incidence of calcospherites. Sommers and Meissner²⁰ found no focal calcification or psammoma bodies in 142 necropsies in cases of carcinoma of the pancreas.

Cameron²¹ observed microliths in neoplasms of pulmonary and gastrointestinal origin; the incidence was low considering the higher frequency of these neoplasms as compared with thyroid carcinomas. In bronchiolar-cell carcinoma of the lung, Smith and associates²² found psammoma bodies in alveoli surrounded by tumor cells in 9 of 20 cases.

Do these peculiar bodies occur in normal tissues? Plaut and Galenson,²³ in studying concretions of the anterior pituitary, mentioned that they had *never* seen concretions in the thyroid glands of fetuses or newborns, and even in adults they were seldom encountered. They recorded a psammoma body found in an otherwise normal renal cortex of a 5-year-old patient.

The uncommon occurrence of calcospherites in the non-cancer-bearing thyroid gland was reported by Klinck,³ Crile and Fisher,⁴ and Winship and Chase.¹⁸ Naylor,²⁴ in a survey of 300 thyroid glands from patients with lymphomas, found no calcospherites. These negative findings in noncancerous thyroid glands are further supported by

Spatz,²⁵ who found no psammoma bodies in a study of 120 thyroid glands from the maternal tissue registry. Hazard and Kaufman²⁶ reported no calcospherites in thyroid glands from 408 necropsies. There was no reference to calcospherites in the necropsy series by Hellwig.²⁷

Inasmuch as calcospherites predominantly occur in malignant neoplasms, in particular in thyroid carcinomas, the questions arise: What are these bodies? Do they have prognostic significance? To the latter question, the answer apparently is "no."²⁸ However, the nature of these bodies is related to the more complex forms of pathologic calcification. The microliths, because of their lamellated character, are separable from the calcific deposits that may be defined as granular or "dystrophic," and this granular type of calcification is frequently found in the thyroid gland. In this instance calcification is a postnecrotic phenomenon. In the formation of the calcospherite, however, this sequence is not a constant one, and frequently, at least morphologically, associated necrosis is the exception.

The calcific bodies appear to be formed for the most part in an intercellular position, gradually increasing in size by the addition of successive layers of lime salts. In some instances they appear to be formed in inspissated colloid, but just as frequently they occur in the absence of colloid. They are not limited to areas containing epithelial cells, but occur with almost equal regularity in dense collagenous stroma. The presence of traces of iron in the bodies is not helpful, since it is known that iron is usually present in calcific deposits. A periodic acid-Schiff-positive matrix or nidus found after decalcification suggests origin from colloid but does not necessarily prove it.

Throughout the studies, we noted focal calcification of macrophages containing lipid and hemosiderin; this suggested that these macrophages might be the nidus for concentric lamellation of minerals. Macrophages were abundant in the papillary neoplasms studied, and also were reported

by Klinck.¹⁷ However, macrophages are also numerous in benign processes in the thyroid gland, although few psammoma bodies are found.

The absence of an inflammatory reaction about the calcospherites places the origin from extruded colloid in a somewhat doubtful category.^{29,30}

Dargent and Guinet³¹ believed that the bodies arose as an edematous bleb in epithelial cells which projected into the lumen of the follicle and ultimately ruptured. The epithelial covering was lost, and calcium was deposited in successive layers, leading eventually to a "calcospherite." Ross and associates,³² in describing similar concretions in adrenal glands of animals, concluded that calcospherites were formed between cells.

Regardless of their origin, descriptions of the microliths have been essentially the same—round, usually laminated, somewhat concentric bodies, varying in diameter from 10 μ to 100 μ , and usually easily distinguishable from other foci of dystrophic calcification.

Although calcospherites occur predominantly in papillary tumors, they may be found in all types of epithelial neoplasms of the thyroid gland, albeit approximately twice as frequently in papillary carcinomas. They are not limited to the thyroid gland, but are present in regional lymph nodes bearing metastases, and in several instances only a solitary calcospherite was present without a metastatic epithelial component in the same plane of section. Since we found no relationship between necrosis and the presence of calcospherites, the latter are not thought to be a manifestation of dystrophic calcification.

Despite the limited knowledge concerning their origin and nature, the psammoma body (microlith, calcospherite, or corpus calcificans) should be included as an aid in the diagnosis of thyroid carcinoma, particularly of the papillary type. Of course, one can find refuge in assuming that there is no benign papillary neoplasm of the thyroid

MICROLITHIASIS AND CARCINOMA OF THYROID

gland (Willis³³), or consider Dunhill's³⁴ work, in which, in a large series of 1,044 thyroidectomies, he found only 2 cases of simple papillary adenoma, 1 of which he concluded might be malignant.

It is appropriate, therefore, to insert Graham's words of caution:

Many pathologists appear to have been so intrigued by the term "papillary carcinoma" that they seem unwilling to admit that there may be such a thing as a benign papilloma of the thyroid, that papilliferous or papillomatous changes do occur frequently in benign adenomata and that similar intra-acinar or intra-cystic changes do occur in otherwise non-tumorous glands.

Summary

In a study of 819 surgically removed thyroid glands, calcospherites were found in 94, or 11.4%.

Carcinomas comprised 207 cases in this study, and calcospherites were present in 84 cases, or 40.5%. This incidence is in sharp contrast to an occurrence of calcospherites in benign conditions of the thyroid gland of only 1.6%. Although one-half of the papillary neoplasms in this series possessed these characteristic bodies, they can occur in any type of carcinoma of the thyroid gland. Calcospherites in thyroid carcinoma may serve as a diagnostic, but not a prognostic, aid, and as such should be added to the various diagnostic criteria employed in the diagnosis of thyroid carcinoma.

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REFERENCES

1. Park, W. W., and Lees, J. C.: The Histology of Cancer of the Thyroid, *Cancer* 8:320-335 (March-April) 1955.
2. Batsakis, J. G.: Calcospherites and Thyroid Carcinoma, *Univ. Michigan M. Bull.* 22:530-532 (Nov.) 1956.
3. Klinck, G. H., and others, in discussion on Klinck, G. H., and Winship, T.: Occult Sclerosing Carcinoma of the Thyroid, *Cancer* 8:701-706 (July) 1955; *Tr. Am. Goiter A.*, 1955, pp. 295-297.
4. Crile, G., Jr., and Fisher, E. R.: Simultaneous Occurrence of Thyroiditis and Papillary Car-

cinoma: Report of Cases, *Cancer* 6:57-62 (Jan.) 1953.

5. Underwood, C. R.; Ackerman, L. V., and Eckert, C.: Papillary Carcinoma of the Thyroid: An Evaluation of Surgical Therapy, *Surgery* 43: 610-621 (April) 1958.

6. Klinck, G. H., and Winship, T.: Psammoma Bodies and Thyroid Cancer, *Cancer* 12:656-662 (July-Aug.) 1959.

7. Graham, A.: Malignant Tumors of the Thyroid: Epithelial Types, *Ann. Surg.* 82:30-44 (July) 1925.

8. Graham, A.: Malignant Epithelial Tumors of Thyroid, *Surg. Gynec. & Obst.* 39:781-790 (Dec.) 1924.

9. Warren, S.: Significance of Invasion of Blood Vessels in Adenomas of Thyroid Gland, *Arch. Path.* 11:255-257 (Feb.) 1931.

10. Warren, S., and Feldman, J. D.: The Nature of Lateral "Aberrant" Thyroid Tumors, *Surg. Gynec. & Obst.* 88:31-44 (Jan.) 1949.

11. Stout, A. P.: Human Cancer: Etiological Factors, Precancerous Lesions, Growth, Spread, Symptoms, Diagnosis, Prognosis, Principles of Treatment, Philadelphia, Lea & Febiger, 1932.

12. Warren, S.: Invasion of Blood Vessels in Thyroid Cancer, Editorial, *Am. J. Clin. Path.* 26: 64-65 (Jan.) 1956.

13. Lahey, F. H.; Hare, H. F., and Warren, S.: Carcinoma of Thyroid, *Ann. Surg.* 112:977-1005 (Dec.) 1940.

14. Ewing, J.: Neoplastic Disease: A Treatise on Tumors, Ed. 4, Philadelphia, W. B. Saunders Company, 1940.

15. Anderson, W. A. D.: Pathology, Ed. 3, St. Louis, C. V. Mosby Company, 1957.

16. Warren, S., and Meissner, W. A.: Tumors of the Thyroid Gland, in Atlas of Tumor Pathology, Armed Forces Institute of Pathology, Sec. IV, Fasc. 14, National Research Council, Washington, D.C., 1953.

17. Klinck, G. H., Jr.: Papillary Tumors of Thyroid Gland, *New York J. Med.* 49:302-305 (Feb. 1) 1949.

18. Winship, T., and Chase, W. W.: Thyroid Carcinoma in Children, *Surg. Gynec. & Obst.* 101: 217-224 (July) 1955.

19. Abrams, G. D., and Tiziani, J. J.: Personal communication.

20. Sommers, S. C., and Meissner, W. A.: Unusual Carcinomas of the Pancreas, *A.M.A. Arch. Path.* 58:101-111 (Aug.) 1954.

21. Cameron, G. R.: Pathology of the Cell, Springfield, Ill., Charles C Thomas, Publisher, 1952.

22. Smith, R. R.; Knudtson, K. P., and Watson, W. L.: Terminal Bronchiolar or "Alveolar Cell" Cancer of the Lung: Report of 20 Cases, *Cancer* 2:972-990 (Nov.) 1949.
23. Plaut, A., and Galenson, E.: Concretions in the Anterior Pituitary Lobe of Human Embryo and Newborn, *Am. J. Path.* 20:223-237 (March) 1944.
24. Naylor, B.: Secondary Lymphoblastomatous Involvement of the Thyroid Gland, *A.M.A. Arch. Path.* 67:432-438 (April) 1959.
25. Spatz, M. S.: Personal communication.
26. Hazard, J. B., and Kaufman, N., cited by Crile and Fisher.⁴
27. Hellwig, C. A.: Thyroid Gland in Kansas, *Am. J. Clin. Path.* 5:103-111 (March) 1935.
28. Rich, C. R.; Batsakis, J. G., and Nishiyama, R. N.: Unpublished data.
29. Ferguson, J. A.: Tissue Reaction to Colloid and Lipoids from Human Thyroid Gland, *Arch. Path.* 15:244-254 (Feb.) 1933.
30. Hellwig, C. A.: Colloidophagy in the Thyroid Gland, *A.M.A. Arch. Path.* 58:151-152 (Aug.) 1954.
31. Dargent, M., and Guinet, P.: Les Tumeurs papillaires du corps thyroïde, *Lyon chir.* 45:543-564 (July) 1950.
32. Ross, M. A.; Gainer, J. H., and Innes, J. R. M.: Dystrophic Calcification in the Adrenal Glands of Monkeys, Cats and Dogs, *A.M.A. Arch. Path.* 60:655-662 (Dec.) 1955.
33. Willis, R. A.: *Pathology of Tumours*, Ed. 2, St. Louis, C. V. Mosby Company, 1953, pp. 602-611.
34. Dunhill, T. P.: Carcinoma of Thyroid Gland, *Brit. J. Surg.* 19:83-113 (July) 1931.

Protection Against Acceleration by Immersion During Hypothermic Suspended Animation

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The exploration of outer space will require the transit of vast distances over periods of time equivalent to geologic eras. This is well beyond the physiologic capabilities of man. A possibility, however, of circumventing the space-time obstacle exists: It is found in Einstein's interpretations of the Lorentz-FitzGerald equations, which, considering the speed of light as the limit, state that, with increasing uniform velocity, time slows (dilates), relative to an observer on earth. This property of uniform velocity begins to assume significance for space travel at a speed approximating that of light itself.¹⁻⁵ With this "relative" clock measuring the passage of time, man could attempt the exploration of outer space if a means could be found of protecting him against the great forces generated in the short time during which such velocities should be attained. This paper describes a step toward such a solution.

Nature has provided the design of an ideal space capsule: the amniotic sac and its fluid. This statement is justified by the fact that the net force (f) created by acceleration (a) upon a submerged homogeneous object of volume V is modified in accordance with the formula:

$$f = V(\delta_1 - \delta_2)a^{0.7} \quad (a)$$

where δ_1 and δ_2 are the specific gravities, respectively, of the submerged object and of the displaced fluid. If $\delta_1 = \delta_2$, then $f = 0$.

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When the hydrostatic pressure generated does not significantly distort the object, acceleration, regardless of its magnitude or duration, should be without effect. If, however, the submerged body is markedly heterogeneous in respect to specific gravity, as are mammals, there is a practical limit beyond which acceleration will cause injury, since $f \neq 0$. As will be shown, such stresses lie beyond those encountered by submerged, hypothermic 8-12-day-old mice, subjected for 15 minutes to a constant rotary acceleration which would result in a hypothetical terminal rectilinear velocity* 7.8% that of light.

The principle of protecting animals and man against acceleration by immersion is not new. Morris, Beischer, and Zarriello⁶ reviewed the various attempts and proposals. Since the advent of the space rocket, Margaria, Gualtierotti, and Spinelli⁶ have demonstrated the protection afforded to fish, frogs, and rats by immersion in water. They found that, without support, 100 G applied

*The force generated by the motion of a centrifuge and acting upon the spinning object is usually expressed in units of G (gravity). The number of G is determined by

$$G = \frac{4\pi^2 r n^2}{980}$$

where r is the radius of spin, n is the number of revolutions per second, and 980 the gravitational constant. This last is defined as the rectilinear acceleration achieved in one second by a body in free fall in vacuum, starting at rest.

Angular acceleration, $a\omega$, is derived from $a\omega = \frac{v^2}{r}$ where v is the velocity of the body in the centrifuge and r the radius of spin. The rectilinear terminal-velocity equivalent of a given value for $a\omega$ is calculated from $v_t = at$, where v_t is the terminal rectilinear velocity produced by acceleration $a = a\omega$ acting over t number of seconds.

to rats for 14 μ sec. was fatal; immersed, the end-point was about 1,200 G. Decelerating an immersed pregnant rat at 10,000 G, they demonstrated survival of its fetuses, although the mother was instantly killed. The authors concluded that "rat foetuses, having no air in their lungs, can survive impacts corresponding to accelerations higher than 10,000 G when the mother is floating in water."⁶

An initial experiment, applying up to 14 G on totally immersed men, has recently been reported.⁹

Morris, Beischer, and Zarriello,⁸ stimulated by the work of Margaria et al.,^{6,7} subjected 12 to 20 gm. mice enclosed in latex tubes containing some air to a rotary acceleration of 1,300 G. They discovered that, immersed or not, maximum survival was 30 seconds. Not reckoned was the time necessary to achieve 1,300 G and deceleration, which consumed about three minutes. When the air was displaced by oxygen, immersed survival rose to a maximum of 90 seconds, while that for the non-immersed animals was raised to 60 seconds. It seemed to the investigators that "at such great G forces, oxygen is of more importance than submersion. When the animal is breathing oxygen, however, submersion offers further protection."

Both Margaria and Morris and their associates used water for their immersions. Since a large amount of air is trapped in fur, it can be shown from the difference in the specific gravities of the mice and water that Morris et al. were exposing their animals to an effective force of approximately 65 G \dagger (for a mouse of 13.7 gm.). This, over a period of 90 seconds, is not lethal. \ddagger The period of apnea, 3.5 to 4.5 minutes,¹⁰ is, however, in the lethal range for normal mice. Unfortunately, this nulli-

fied the possibility of demonstrating a more significant anti-G effect of immersion.

It is apparent that some means must be employed for separating the effects of the mechanical stress of G from the eventual anoxia associated with the immersion, and the splinting effects upon the thorax of the G and the hydrostatic pressure. To achieve this, use was made of the well-established ability of the infant mouse to survive immersion in ice water for periods up to one hour. During this time there is cessation of respiration, heart beat, and metabolism—in effect, hypothermic suspended animation.^{11,12}

Methods and Results

The mice, chosen for size, were from a random-bred strain of white Swiss mice. They weighed from 2.7 to 4.8 gm. and varied in age from 8 to 12 days. The smallest was free of grossly visible hair, and the largest was covered with a fine, short fuzz.

To relate viability to duration of hypothermic suspended animation, the following experiment was performed: Mice were placed in finger cots, which were immersed in ice water until all visible motion ceased. This took about five to eight minutes. The cots were then filled with isotonic NaCl solution (4 C) and tied off. The enclosed immersed mice were placed in a beaker of ice water and kept in a refrigerator at 4 C. The animals were removed, usually in pairs, at 15-minute intervals, as indicated in the Table. Reanimation was 100% at the end of 15 minutes and 0.0% at 60 minutes. Intermediate survival rates were found between these extremes.

As a preliminary, the tolerance of infant mice to G stress was determined by centrifuging § unsupported, cooled mice (4-8 C), in pairs, for 5, 10, and 15 minutes. Thirteen pairs of mice were

§ International Refrigerated Centrifuge, Model PR 2, was used throughout the experiment.

Declining Ability of Infant Mice to Survive Increasing Periods of Immersion in Ice Water or Isotonic Saline at 4 C

Duration of Immersion, Min.	No. Revived	No. Died	Totals
15	5	0	5
30	4	3	7
45	2	5	7
60	0	6	6
	—	—	—
Totals	11	14	25

\dagger It was found that a 13.7 gm. mouse whose fur was freed of air remained suspended in a 7% solution of NaCl at about 20 C.

\ddagger We have centrifuged unsupported mice of about 11-12 gm. at approximately 100 G for 90 seconds with survival.

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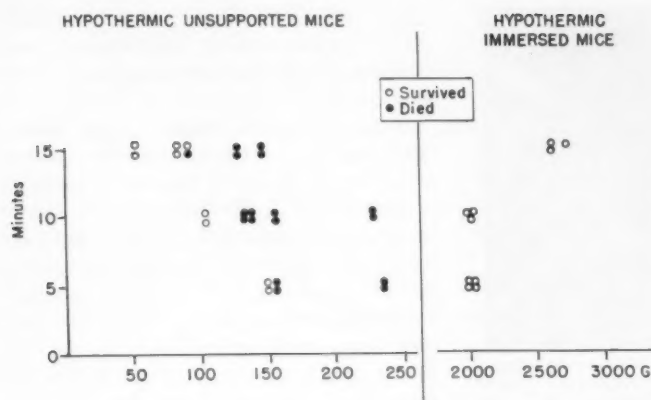


Fig. 1.—The natural high tolerance to G stress of 8-12-day-old mice is illustrated on the left. With increasing prolongation of the spin, tolerance decreases. The magnitude of immersion protection (right) is circumscribed by the limits of the refrigerated centrifuge. All of the animals were spun at the maximum speed of the machine, 3,500 rpm.

subjected to stress varying from 53 to 236 G. Prior to centrifugation they were inserted into individual finger cots and immersed in ice water for six to eight minutes. A control mouse accompanied each of these pairs through all procedures except the centrifugation. The control was placed in a cooled beaker at the bottom of the centrifuge and was removed for reanimation after the experimental pair, thus being exposed to a longer cooling and apneic period. If the control mouse survived the hypothermia (only 1 of 19 died), then death of one or both of the spun mice was attributed to the G stress. A spin was considered fatal if the mouse did not respond to body warming, or if, after responding, it died within two hours, the minimum period of observation.

The resistance to G stress of unsupported hypothermic mice is compared with that of immersed hypothermic mice in Figure 1. Survival of the unsupported mice was inversely related to duration and intensity of stress. The mice died after 5 minutes of 150 G, 10 minutes of 135 G, and 15 minutes of 90 G. In contrast, the immersed mice survived.

The conditions of immersion were varied with the duration of centrifugation. In the first experiment three mice, weighing respectively 3.7, 3.8, and 4.2 gm., were individually dry-cooled, the air in the finger cots being displaced by oxygen. When inanimate, they were transferred from the finger cots to centrifuge buckets filled with 5% NaCl brine, a solution which had been found to have approximately the same specific gravity as the mice. They were thereupon centrifuged for five minutes at the maximum speed of the machine, 3,500 rpm. The estimated radius of rotation was 15 cm. All responded to rewarming, and when given to a foster mother, suckled and grew normally. These three were protected against five minutes of 2,000 G. In contrast, unsupported mice exposed for five minutes to 154 G died. This

interval, for both groups, does not include the time consumed in reaching maximum velocity and deceleration, which, in the light of further experience, may be neglected.

In the second experiment, three immersed mice were spun for 10 minutes. Because the 5% brine was irritating to the hands and in view of the infant mouse's delicate skin, as well as the appearance of foam at the nostrils of the revived five-minute mice, the finger cots were filled with precooled isotonic saline solution. These were tied off; the free rubber was trimmed away, and cot with mouse immersed in a NaCl solution adjusted to suspend the "amnion." (This term is used because of the appearance of the "inanimate" mouse floating in its transparent envelope.) Thus encapsulated, two mice, of 3.7 and 3.6 gm. each, were spun at 3,500 rpm. Upon delivery from their "amnions," they responded promptly and completely to rewarming and have thrived. They were protected for 10 minutes against 2,000 G. The strain upon the mice must have been reduced by the immersion to less than the stress of 136 G to permit survival (Fig. 1), a 15-fold protection whose maximum could not be determined because of the limitations of our centrifuge.

Since preparation of the mice consumed from 8 to 10 minutes, and starting and stopping of the centrifuge added another 3 minutes, a 15-minute centrifuge run required about 26 to 28 minutes from the initial cooling immersion to removal from the centrifuge. A 30-minute run extended this period to 40 minutes. With experience this was reduced; however, it was decided to make the 15-minute run the maximum, since the longer period of hypothermia killed mice.

Seven mice were encapsulated and prepared as in the preceding experiment and spun for 15 minutes. One after the other dead mice, with little external evidence of injury except for moderate hemorrhagic suffusions about the snout, were ex-

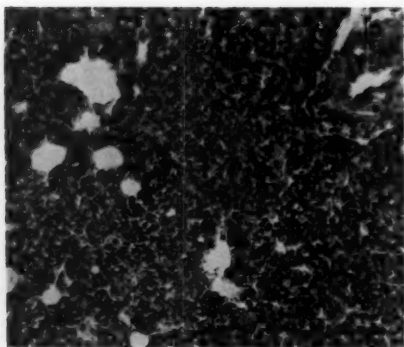
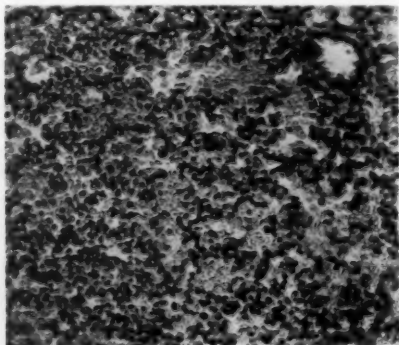


Fig. 2.—The virtually complete pulmonary atelectasis illustrates the effect of hydrostatic pressure upon the thorax and lungs of the immersed mouse.

tracted from their amnions. One, upon rewarming, gasped a few times, but circulation was not restored, and it died. Eleven more mice were thereupon centrifuged without latex capsules in 5% NaCl solution. These revealed no external evidence of injury. The best result was obtained with a 4.4 gm. animal spun at 2,000 G; this mouse revived and survived for about one-half hour, with poor respirations and circulation. In all, 18 consecutive unsuccessful attempts were carried out. The last four animals that died were autopsied, and in three cases the lungs were found to be uniformly atelectatic. The fourth, the half-hour survivor described above, showed mottled lungs, due to widely disseminated focal atelectasis. No gross lesions were found in the other organs or brains.

Histologic examination of the tissues of the four mice confirmed the presence of atelectasis (Fig. 2) and revealed an occasional pulmonary hemorrhage (Fig. 3). In no case were these large enough to have

Fig. 3.—The intra-alveolar pulmonary hemorrhage is characteristic of the small focal hemorrhages seen in some of the nonsurvivors.



caused perceptible sequelae. In addition, some focal emphysema (Fig. 4) was noted to alternate with the atelectasis in all of the lungs.

Because of these results, it was speculated that the hydrostatic pressure transmitted to the heart or respiratory muscles or both might be the cause of the failure to survive. Immersed free in the centrifuge bucket or enclosed in the latex "amion," the mice were subjected to the full pressure generated by the angular acceleration. Since they were suspended in NaCl solution at a radius of 15 cm. (it is assumed that the midpoint, a depth of 5 cm., best approximates their position) the hydrostatic pressure would be around 10 kg/sq. cm. (140

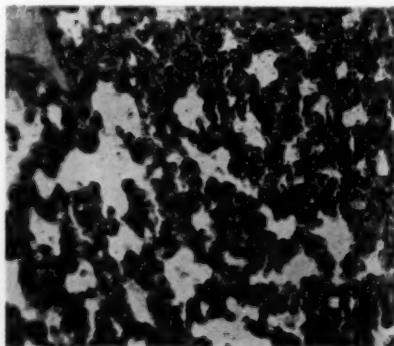


Fig. 4.—The focal acute emphysema superimposed upon atelectasis, presumably due to the inability of some of the animals to reexpand their lungs is illustrated by a mouse which survived for one-half hour after 15 minutes of 2,600 G. Deep, gasping respirations characterized the entire post-centrifugation period.

lb. sq. in. 9.6 atm.). It had been determined (Fig. 1) that 10 minutes of this pressure at 4-8 C could be tolerated by the mice. Whether 15 minutes was as harmless was not known. It was decided, therefore, to eliminate most of it by enclosing the animals in a rigid plastic capsule that would diminish the hydrostatic pressure to that exerted by the water within the capsule, a column of not more than 0.5 cm. height. The hydrostatic pressure under these circumstances is 1.3 kg/sq. cm. Accordingly, two mice, of 3.3 and 3.7 gm. each, were

spun in a small plastic barrel || filled with 5% NaCl solution at 3,500 rpm for 15 minutes, at a radius of 18.6 cm. This is equivalent to 2,600 G (Fig. 1). Both animals revived promptly and are thriving. These two animals tolerated a G load about 29 times the lethal level (Fig. 1).

Equally good results should be expected from centrifugation in brine at a depth just sufficient to cover the mouse. This was done with a 3.6 gm. animal. It was spun for 15 minutes, at a radius of 19.5 cm., resulting in a force of 2,700 G (Fig. 1). The animal was revived with ease and has survived.

Comment

Hydrostatic pressure in the absence of circulation serves as an ideal G suit, since the only pressure produced in the cardiovascular system is that generated by the acceleration. As this is likewise the controlling factor in the level of the environmental hydrostatic pressure, no significant load is placed upon the circulatory system when the mouse is accelerated. This is confirmed by the rapid and effective resumption of cardiovascular function upon rewarming and the absence of any recognizable lesions, aside from an occasional petechial hemorrhage in the lungs of two of four autopsied mice and focal emphysema in all.

The rapid deceleration of the mice, in about two minutes, reduced the hydrostatic pressure from 10 kg/sq. cm. to 5 gm/sq. cm. This is identical with surfacing from a depth of 325 ft. of water. The blood plasma and tissue fluids of a diver at this level would contain considerably larger amounts of air than normal, owing to the increased air pressure applied to the respiratory tract in countering hydrostatic pressure. Such rapid deceleration would produce fatal "caisson disease." In contrast, the blood plasma and tissue fluids of the mouse could contain no more air than was present in the lungs when centrifugation was begun. This

proved to be insignificant, as evidenced by the absence of appropriate lesions. Since the gaseous phase of the compression was not harmful, it may be ignored for the purpose of further discussion.

Little is known of the effects of hydrostatic pressure upon mammals, Cattell¹³ stated the reason: "Experiments in the influence of hydrostatic pressure on mammals are, of course, not possible on account of the necessary pressure of a gaseous phase." The biologic effect of pressure on a number of nonmammals has been studied.¹³⁻¹⁵ They are related to changes of molecular shape, elongated \rightleftharpoons globular—with alterations in gel-sol equilibria; rearrangement of molecular surface groups, causing changes in bonding ability, solubility, and electrostriction phenomena; denaturation of proteins; inactivation of enzymes, etc. Reflecting a variety of these alterations, acting alone or together, are highly significant changes of protoplasmic viscosity.

The temperature at which a given pressure acts may be extremely important.¹⁴⁻¹⁶ Landau and Marsland¹⁶ observed the effects of pressure-temperature relationships on the cardiac rate of tissue explants of tadpole heart. They noted that, at atmospheric pressure with falling temperature, the rate declined exponentially between 12 and 5 C. When 4,000 lb. sq. in. (ca. 281 kg/sq. cm.) was applied over the same temperature range, it increased the rate retardation effect. Johnson, Eyring, and Polissar¹⁴ generalized that the greater the difference between physiologic and experimental temperature of a tissue the greater the effect of a given pressure. Since mammalian tissues function normally at 37 to 38 C and hypothermic suspended animation requires temperatures below 10 C, it is readily appreciated that the effects of small pressures may be significantly amplified. The mechanism whereby 10 kg/sq. cm. pressure acting upon hypothermic mice for 15 minutes causes their death has yet to be elucidated.

In the experiments the heterogeneity of the tissue densities (bone, specific gravity 3.00; aerated lung, 0.45; the solid organs

|| Kitty-in-the-Kegs, Child Guidance Toys, Archer Plastics Co., New York.

and tissues in general, about 1.05^8) caused no lesions. Hydrostatic pressure quickly collapsed the flexible infantile thorax, and the lungs became almost completely atelectatic (Fig. 2). The density approached (but probably never reached) that of the solid tissues; thus little or no injury was sustained.

It is not known what force would cause infant mouse bone to slip its muscle and ligament attachments and migrate in accordance with its flotation value. Since bones are immersed in soft tissues, the accelerative forces are acting against their weight minus that of the soft-tissue volume they displace times the soft-tissue specific gravity; therefore, by formula (a), the force tending to displace the bone is reduced by about 64%.

Considering the above, and the great adhesiveness between bone and its encompassing soft tissues, it would not be surprising if immersion could protect against 15 minutes of a multiple of 2,700 G.

Concerning the time dilation effect of constant velocity, the mouse protected against 2,700 G for 15 minutes was buffered against a force ($a_0 = 26.1 \text{ km/sec}^2$), which, if equated with rectilinear acceleration, would have given it a terminal straight-line velocity of 23,500 km/sec. (14,500 miles/sec). This, according to the Lorentz-FitzGerald formula,

$$t' = t \sqrt{1 - \frac{v^2}{c^2}} = 1\% \quad (b)$$

where t' is the dilated time; t , geodesic time; v , the mouse's hypothetical velocity, and c , the speed of light, would have resulted in a slowing of his time relative to that of earth by 1%.

All of the mice spun at or above 2,000 G showed disturbances of equilibrium. They fell toward one side, had difficulty in righting themselves, and, when they walked, described circular pathways.

The mice described in the above experiments, when observed for several weeks, showed restoration of their equilibrium

after opening of their eyes. These and other appropriate data will be the subject of a separate report.

Summary and Conclusion

When baby mice 8-12 days old in hypothermic suspended animation are centrifuged, the mechanical effects of acceleration upon the tissues are readily separated from the effects upon function, such as respiration, cardiovascular dynamics, and metabolism. Under these circumstances the highly effective protection against acceleration by immersion, to the limits of our apparatus (ca. 2,700 G), is readily demonstrated.

Immersion during acceleration introduces a new mechanical factor, hydrostatic pressure. Temperature changes may under given conditions reinforce or reduce the physiologic effects of pressure. Under the conditions of this experiment, a pressure up to 10 minutes of approximately 10 kg/sq. cm. at 4 to 8 C is successfully tolerated. When this is prolonged for 15 or more minutes, it is lethal.

It is shown that, when immersed, the mice can be protected against an accelerative stress, which, when translated into rectilinear velocity, would, at the end of 15 minutes impart a constant speed of about 23,500 km. per second (14,500 miles/sec.). At such constant velocity a relativistic time dilation of 1% would occur.

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REFERENCES

1. Dingle, H.: *Nature*, London 177:782, 1956.
2. McCrea, W. M.: *Nature*, London 177:782, 1956.
3. Fremlin, J. H.: *Nature*, London 180:499, 1957.
4. Dingle, H.: *Nature*, London 180:500, 1957.
5. McMillan, E. M.: *Science* 126:381-384, 1957.
6. Margaria, R.; Gualtierotti, T., and Spinelli, D.: *J. Aviation Med.* 29:433, 1958.
7. Margaria, R.: *J. Aviation Med.* 29:855, 1958.
8. Morris, D. P., Jr.; Beischer, D. E., and Zariello, J. J.: *J. Aviation Med.* 29:438, 1958.

PROTECTION AGAINST ACCELERATION

9. Bondurant, S.; Blanchard, W. G.; Clarke, N. P., and Moore, F.: *J. Aviation Med.* 29:872, 1958.
10. Hicks, S. P.: *A.M.A. Arch. Path.* 55:302, 1953.
11. Adolph, E. F.: *Am. J. Physiol.* 166:75, 1951.
12. Fairfield, J.: *Am. J. Physiol.* 155:355, 1948.
13. Cattell, M.: *Biol. Rev.* 11:441, 1936.
14. Johnson, F. H.; Eyring, H., and Polissar, M. J.: *The Kinetic Basis of Molecular Biology*, New York, John Wiley & Sons, Inc., 1954, pp. 286-368.
15. Marsland, D.: *Scient. Am.* 199:36, 1958.
16. Landau, J., and Marsland, D.: *J. Cell. & Comp. Physiol.* 40:367, 1952.

Liposarcoma Developing in a Lipoma

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Primary liposarcomas are rare in the subcutaneous tissue and practically never originate in simple lipomas. In fact, we found only two such cases reported in the English literature. Stout,⁴ in 1944, reported among several cases of liposarcomas only one arising in a lipoma which was retroperitoneal in location (Case 21). Wright,⁸ in 1948, reported a case of a liposarcoma arising in a subcutaneous lipoma and reviewed the literature from 1881. He accepted Stout's Case 21 and excluded all other previously reported cases, mainly on the basis of poor documentation and inconclusive histologic evidence. In 1952, Sternberg,⁹ reported a case similar to Wright's and stated that Stout, after reviewing his Case 21, was no longer convinced that it represented this entity. Stout⁶ maintained that most liposarcomas probably arise *de novo*, and not from preexisting lipomas or following trauma.

We believe that the following case may represent the third authentic report of this rare entity.

Report of a Case

The patient, a 79-year-old Negro woman, was admitted to Freedmen's Hospital on Oct. 29, 1958, for the removal of a right scapular mass that had been present for eight years. Two years before admission she had noticed a gradual increase in its size. Shortly before admission she complained of paresthesias over the mass. She denied previous trauma.

Physical examination revealed an asthenic, but otherwise healthy-appearing elderly woman with a firm, nontender, freely movable mass, measuring

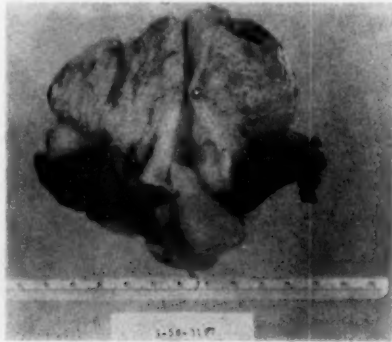
10.5×7×5 cm., located subcutaneously just above the right scapula. There were no ulcerations, cutaneous infiltrations, or enlarged regional lymph nodes. The remainder of the examination, including chest x-rays, routine hematology, and urinalysis, was within normal limits. The preoperative diagnosis was simple lipoma, and on Oct. 30 the mass was removed under local anesthesia. The mass was well circumscribed, confined to the subcutaneous tissue, and not adherent to the skin or underlying fascia. It was removed intact and was thought to be a simple lipoma. Her postoperative course was uneventful, and she was discharged on Nov. 3, 1958, for follow-up by her private physician. Because of the histological findings, to be given below, a total of 11,000 r was given locally over a period of seven weeks. To date, nine and one-half months postoperatively, there has been no evidence of local recurrence or metastasis.

Pathologic Findings

Gross Examination

The specimen, received in formalin, consisted of a firm, encapsulated, somewhat lobulated, ovoid mass of yellow adipose tissue, which measured 10×8×5 cm. It was completely confined to the subcutaneous tissue. The upper surface was covered by an ellipse of skin, which measured 13.5×3×0.5 cm., while the undersurface was flattened and showed areas of white connective tissue (Fig. 1). On cut surface, the peripheral part of the tumor,

Fig. 1.—Gross specimen showing the encapsulated tumor mass covered by an ellipse of skin.



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From the Departments of Pathology and Surgery, Howard University College of Medicine and Freedmen's Hospital.



Fig. 2.—Cross section of the tumor mass showing the centrally located circumscribed myxomatous area.

immediately beneath the capsule, was seen to consist of lobules of fat with the appearance of a typical lipoma. The central part, measuring $5.5 \times 5 \times 4.5$ cm., was different, however, being well circumscribed, firm, translucent, grayish-white, and myxomatous in appearance (Fig. 2).

Microscopic Examination

Representative sections were taken from both parts of the tumor, including sections through the capsule and skin. The sections were stained by the hematoxylin and eosin and periodic acid-Schiff procedures. Frozen sections were stained by the oil red O fat stain.

The central part of the tumor had a varied cellular pattern and contained both

well- and poorly differentiated areas. The well-differentiated area showed fully developed fat intermingled with myxoid tissue containing stellate lipoblasts (Fig. 3). The stellate or spindle lipoblasts contained tiny fat vacuoles in their cytoplasm, as demonstrated by oil red O fat stains (Fig. 4). A few cells resembling "signet-ring cells" were noted. The poorly differentiated area was more extensive than the well-differentiated area. The fat was poorly differentiated and was associated with myxoid areas. Present in this area were extremely bizarre giant cells, containing large, irregular, hyperchromatic, and pyknotic nuclei. Many of the giant cells contained as many as three nuclei. Lipid was demonstrated in the vacuoles of the cytoplasm, which in some instances caused indentations of the nuclei. Many of the unicellular lipoblasts were anaplastic, and a few of these showed mitoses (Fig. 5). Scattered throughout the area were large lipoblasts with congeries of rounded vacuoles in their cytoplasm. The nuclei were multilobulated, suggesting the appearance of brown fat. Notched nuclei were noted in many of these lipoblasts (Fig. 6). Almost all of the cells described appeared viable. There was moderate vascularity, and no areas of infarction or necrosis were seen.

Fig. 3.—Microscopic section of the central part of the tumor showing poorly differentiated fat cells and myxoid tissue containing stellate cells. Hematoxylin and eosin stain; reduced to 89% of mag. $\times 430$.

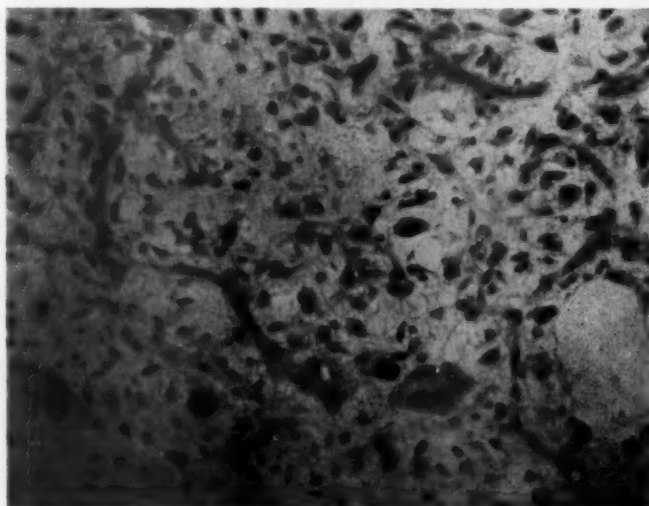
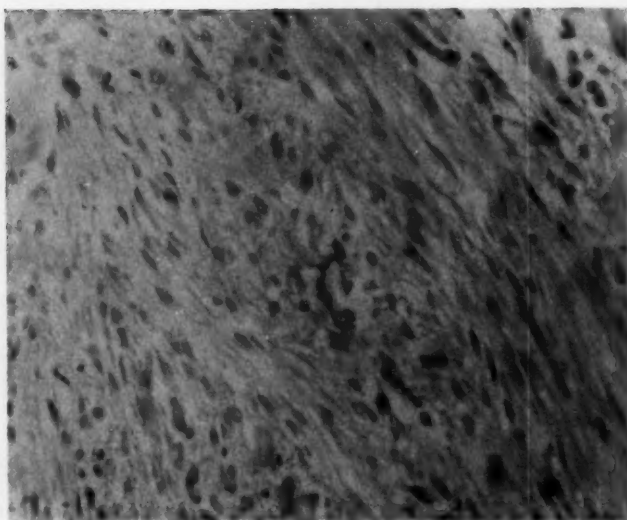


Fig. 4.—Microscopic section showing spindle or stellate lipoblasts. Periodic acid-Schiff stain; reduced to 86% of mag. $\times 100$.



Only occasional cells showing karyolysis of the nuclei were noted.

The malignant cells described were circumscribed by a small, thin layer of fibrous connective tissue, which was infiltrated by a small focus of malignant cells (Fig. 7). The peripheral part of the tumor consisted of mature fat cells, having a "chicken wire" configuration and containing occasional notched nuclei. This portion was completely surrounded by dense fibrous connective tis-

sue, forming a capsule. A few scattered malignant cells were noted invading the interstices of the lipomatous part. However, malignant cells were not seen in the peripheral parts of the lipomatous tumor, the capsule surrounding the entire tumor, or the skin. Vascular invasion was not seen.

Comment

Lipomas are common tumors usually found in the subcutaneous tissue, whereas

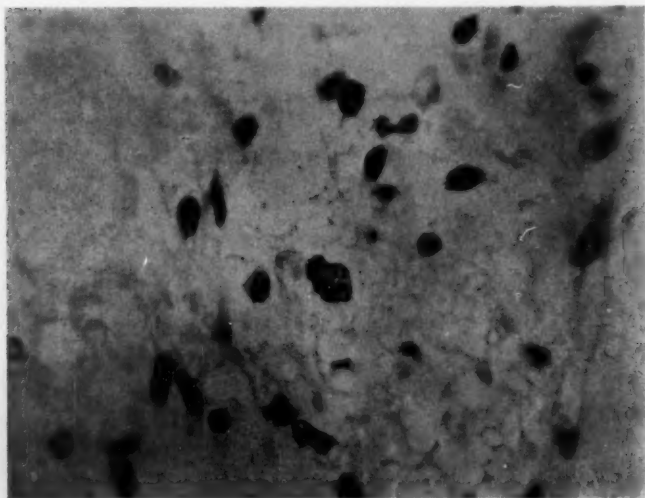


Fig. 5.—Photomicrograph showing mitosis in a unicellular lipoblast. Hematoxylin and eosin; reduced to 89% of mag. $\times 430$.

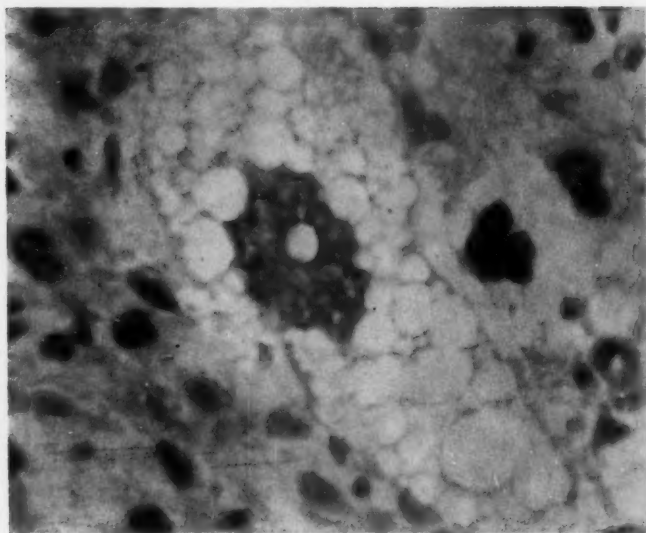
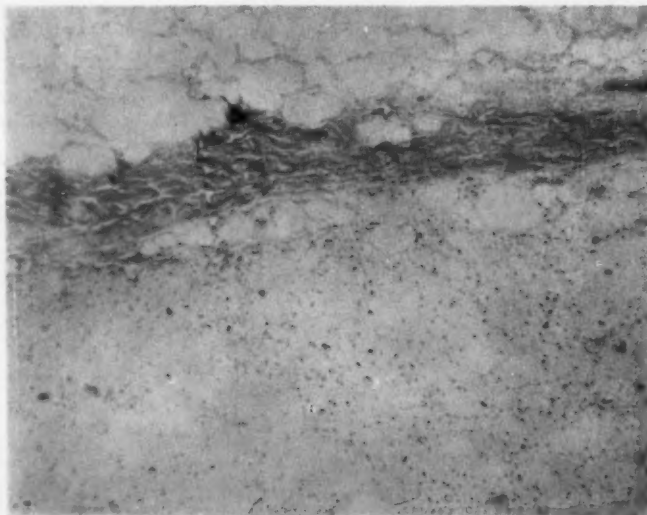


Fig. 6.—Photomicrograph demonstrating the notched nucleus and bizarre giant cells. Hematoxylin and eosin; reduced to 75% of mag. $\times 970$.

liposarcomas are rare and most commonly confined to the deep interstitial fat tissues of the thigh, popliteal space, gluteal region, and retroperitoneum. The gross findings of the malignant lipomatous tumors are characterized by unusual variations of texture or color and the frequent occurrence of myxomatous areas. As stated by Willis,⁷ a liposarcoma arises from lipoblasts. Histologically it usually reproduces the appearance of embryonal fat, either of the myxoid

or of the brown-fat variety. Liposarcomas may be well- or poorly differentiated. The well-differentiated form may be composed of (a) fully developed adult fat, (b) myxoid tissue with stellate cells, (c) spindle or stellate lipoblasts containing vacuolated cytoplasm, and (d) signet-ring cells. This form of liposarcoma usually does not metastasize but may infiltrate locally and recur if not adequately removed. The poorly differentiated type is of the myxoid variety,

Fig. 7.—Microscopic section showing a thin layer of fibrous connective tissue separating the malignant area from the lipomatous area. A small focus of malignant cells is seen in the upper right corner. Hematoxylin and eosin; reduced to 82% of mag. $\times 50$.



containing poorly differentiated fat, bizarre giant cells, and, less commonly, rounded vacuolated lipoblasts of abnormal brown fat. This type may metastasize.

Unna⁶ described the peculiar notched nucleus in 1895, and Rabl² later stated that it occurred only in fat cells regardless of immaturity. Plaut¹ states that this nuclear characteristic may be used for differentiating true fat from lipid-filled cells (pseudo-fat cells). To date this concept has not been contradicted.

The case presented here is unusual but similar to those published by Wright and Sternberg, on the basis that it originated in a subcutaneous lipoma, obviously of long standing, and was surrounded by a well-defined fibrous capsule. The histological findings were also consistent with those described by the two named investigators.

The three cases appear to represent a heretofore unrecognized entity characterized by the following points: (1) The peripheral parts of the tumors represent a simple lipomatous tumor surrounded by a connective tissue capsule; (2) the central areas were liposarcomas, as evidenced by the presence of lipoblasts, bizarre giant cells, myxomatous areas, positive fat reactions, and, in our case, notched nuclei; (3) the malignant tumors showed exodric growth, as evidenced by foci of infiltration of malignant cells out into the lipomatous part; (4) the malignant cells were not seen in the periphery or the capsule of the lipomas; (5) the sarcomas originated in the lipomas, as evidenced by their central location and the absence of further spread or metastases in spite of the long-standing history of the original tumors.

From this study, we feel that a careful pathologic examination of all lipomas is indicated.

Summary

A liposarcoma arising in a subcutaneous lipoma of eight years' duration is presented. The sarcoma was confined to the central part of the lipoma. Recurrence has not been noted following nine and one-half months' observation. Only two similar cases were found in the English literature.

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Addendum

The patient was reexamined during the month of February, 1960. There was no evidence of local recurrence or metastases to regional lymph nodes. This was a total of 16 months after surgery.

REFERENCES

1. Plaut, A.: The Notched Nucleus of the Fat Cell ("Unna's Lochkern"), *J. Mt. Sinai Hosp.* 24:1112-1120, 1957.
2. Rabl, H.: Über die Kerne den Fettzellen, *Arch. mikro. Anat.* 47:407, 1896; cited by Plaut.¹
3. Sternberg, S. S.: Liposarcoma Arising in a Subcutaneous Lipoma, *Cancer* 5:975-978, 1952.
4. Stout, A. P.: Liposarcoma: The Malignant Tumor of Lipoblasts, *Ann. Surg.* 119:86-107, 1944.
5. Stout, A. P.: Tumors of Soft Tissues, in *Atlas of Tumor Pathology*, Sect. 2, Fasc. 5, Washington, D.C., Armed Forces Institute of Pathology, 1954.
6. Unna, P. G.: Zur Kenntnis den Kerne, *Monatsh. prakt. Dermat.* 20:597, 1895; cited by Plaut.¹
7. Willis, R. A.: *Pathology of Tumors*, Ed. 2, St. Louis, The C. V. Mosby Company, 1953.
8. Wright, C. J. E.: Liposarcoma Arising in a Simple Lipoma, *J. Path. & Bact.* 60:483-487, 1948.

Effect of Partial Hepatectomy on Mitosis Rate in CCl₄-Induced Liver Damage of Parabolic Rats

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As early as 1896 Podwyszozi¹ described the process of tissue regeneration in liver, the histological proof of which was later provided by Ponfick² and by von Meister.³ Interest in this regeneration was further stimulated when it was shown that the liver, after having been reduced to 30% of its former size by partial hepatectomy, could regain its former size in a few days, with reconstitution of histological structure in about two weeks. The most rapid segment of this growth took place within the first 48 to 72 hours. The study of this regeneration interested both biochemists and histologists.⁴⁻¹³ Further, Bucher et al.¹⁴ have shown that when two normal animals are united parabiotically, the regenerating liver, after partial hepatectomy of one partner, can definitely influence the mitotic rate in the liver of the other.

This study was initiated with the following objectives: (1) to repeat the work of Bucher et al.,¹⁴ and (2) to attempt to transmit the mitotic impulse to the liver of the nonhepatectomized parabiotic partner, which was previously treated with carbon tetrachloride so as to induce liver damage.

Materials and Methods

White Wistar rats, all females weighing 120 to 150 gm., from an inbred strain of our own stock, were used for these experiments. For parabiosis only litter mates were paired.

Liver Damage.—Owing to the wealth of material and reference standards, it was found very convenient to use carbon tetrachloride as a hepatotoxic substance. In preliminary experiments it was

established that single doses of 0.1 cc. of carbon tetrachloride, diluted in 0.4 cc. of liquid petrolatum U.S.P. (paraffin oil), were too weak. However, 0.125 cc. of carbon tetrachloride in 0.375 cc. of liquid petrolatum proved to give enough and more constant damage. Further experiments have shown that the liquid petrolatum alone, given in repeated doses intraperitoneally, was not capable of producing a mitotic impulse in the normal liver. Also, in the early experiments with repeated intraperitoneal injections of carbon tetrachloride-liquid petrolatum mixture, no mitotic impulse in the damaged liver was found.

Colchicine.—The mitotic activity was measured by means of the Dustin¹⁵ reaction, which had proved to be reliable also in Studer's¹⁶ experiments. Because of the toxicity of colchicine and because the animals were weakened by the procedure, some rats died before termination of the experiment. In our results, however, only animals were considered that lived at least six hours after the injection of colchicine. They were killed at nine and one-half hours after the injection.

Parabiosis.—The procedure carried out here was a slight modification of the method of Bunster and Meyer.¹⁷ This method was chosen over that used by Sauerbruch and Heyde¹⁸ (originally from Bert¹⁹) because the latter, the "open celiac-anastomosis" method, involves the possibility of herniation and intestinal strangulation.

We then had a physiological unit consisting of two animals with one blood supply. The choice of animals was based on the work of Bickel and Holm-Jensen,²⁰ Van Dyke et al.,²¹ and Huff et al.,²² who showed that animals from the same litter established the best "crossed" circulation. Van Dyke et al. demonstrated that the establishment of this blood supply takes only three to four days. However, since in our experiments both animals were not "normal" at the time of operation and since another operative procedure was to be performed, we allowed 10 days for the completion of the "cross" circulation.

Hepatectomy.—We used the method as described by Ingle,²³ with slight modifications.

Histological Preparation and Methods of Counting the Mitoses.—On completion of the experiment the animals were killed by ether narcosis. Specimens from one, usually two, sites in the liver were

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For details on this study, see Hurowitz.²⁴

Department of Experimental Medicine, F. Hoffmann-LaRoche & Co., Ltd., Basle, Switzerland (Dr. Studer).

Summary of Results

Group A Parabiosis + Partial Hepatectomy + Colchicine				Group B Carbon Tetrachloride + Parabiosis + Partial Hepatectomy + Colchicine				Group C Carbon Tetrachloride + Parabiosis + Colchicine			
Mitotic Rate				Mitotic Rate				Carbon-Tetrachloride-Induced Damage			
Pair No.	Partially Hepatec- tomized Partner	Undamaged Partner	Pair No.	Partially Hepatec- tomized Partner	Partner with Carbon-Tetrachlo- ride-Induced Liver Damage	Degree of Liver Damage (0 to +++)*	Rat No.	Mitotic Rate	Degree of Liver Damage (0 to +++)*	Mitotic Rate	Degree of Liver Damage (0 to +++)*
1	749	211	1	295	47	(+)	1	1	(+)		(+)
2	882	9	2	876	3	++	2	0	++		++
3	1290	4	3	684	4	++	3	0	++		++
4	706	211	4	391	0	+	4	0	++		++
5	451	519	5	330	1	+	5	0	++		++
6	890	1	6	769	0	+	6	0	++		++
7	128	55	7	636	1	++			++		++
8	638	91	8	443	2	++			++		++
			9	318	3	++			++		++
			10	924	1	++			++		++
			11	693	0	+			++		++
			12	1337	15	+			++		++
			13	1054	109	++			++		++
			14	209	5	+			++		++
			15	515	1	++			++		++
			16	1180	0	++			++		++
			17	608	1	++			++		++
			18	205	1	++			++		++

* Intermediate degrees of damage are signified by parentheses (+).

taken for histological examination and placed in Bouin's solution and formalin, respectively; 7 μ sections were cut and stained in the usual way with hematoxylin and eosin. The counts were made as follows: Magnified $\times 150$ (ocular 6 \times ; objective 20 \times ; and tube 1.25), the mitoses in 30 neighboring microscopic fields were counted in each of the two specimens. The average of the 60 fields (in Group C only 30 fields) represented the mitotic rate of that liver. The decision as to whether a cell was in active mitosis or not depended on the characteristic arrangement of the chromatin and the enlargement of the cell with a homogeneous lightening of the stain of the cytoplasm.

Experiments and Results

Group A.—Thirteen pairs of animals (litter mates) were united parabolically, and 10 days later one partner was partially hepatectomized. Forty hours later colchicine (1 μ g. per gram of body weight) was injected intraperitoneally. Five pairs, which lived only four to five and one-half hours after this injection, were not included in the final results. The average mitotic rates (from 30 neighboring fields at $\times 150$) for each of the eight animals with normal livers, which were killed eight to nine and one-half hours after the colchicine injections, are given in the Table. They show that in every case there is a large increase in mitosis in the partially hepatectomized liver.

The nonhepatectomized partners, however, reacted differently: In Rats 1, 4, and 5 the increase in mitosis is evident. In Rats 7 and 8, we have tried to show statistically whether or not there exists the possibility of a mitotic impulse. In Rats 2, 3, and 6 a mitotic impulse was not demonstrable.

Group B.—Twenty pairs of animals were included in the experiments. One member of each pair received an intraperitoneal injection of a mixture of 0.125 cc. of carbon tetrachloride and 0.375 cc. of liquid petrolatum three times each week, until a total of 10 such injections were given. Three days after the last injection the animals were united parabolically. Ten days after this operation the animal that had not received the carbon tetrachloride was partially hepatectomized; 40 hours later each animal

was given an injection of colchicine (1 μ g. per gram of body weight) intraperitoneally. In this series all the animals survived the six-hour limit, and those still alive after nine hours were killed. However, two pairs could not be considered in the final results. In one pair the mitosis could not be attributed with certainty to the liver cells, and in the other the partially hepatectomized partner presented an unusual picture, in that the mitotic rate was only about one-fortieth to one-tenth that of the other animals of the series.

Of the 18 pairs which thus remained of Group B (Table), the partially hepatectomized animals showed the usual increase in mitosis. Of the animals that received carbon tetrachloride in the preliminary treatment, we found an increase in mitosis in Rats 1, 12, and 13. The degree of carbon-tetrachloride-induced liver damage in these ranged from + to +++, using the designation of 0 to +++ to grade the liver damage, as estimated by histological examination.

The liver damage, in the stages examined here, consisted of an increase in connective tissue and a disappearance of central lobular parenchymal cells.

Control Group C.—This group consisted of six pairs; one partner of each pair received the 10 intraperitoneal injections of carbon tetrachloride in liquid petrolatum, as described for Group B. Three days after the last injection the animals were united parabolically, and twelve days later, nine and one-half hours after intraperitoneal injection of colchicine (1 μ g. per gram of body weight), the animals were killed.

In the partners with tetrachloride-induced liver damage there was no demonstrable increase in mitosis. Again, the degree of liver damage ranged from + to +++.

Comment

The results of Bucher et al.¹⁴ indicate that an impulse which stimulates mitosis in liver cells can be transmitted from a partially hepatectomized rat to its healthy parabiotic

parther: A comparison of Groups A and B (Table) will answer the question whether this impulse elicited by partial hepatectomy can also be transmitted to a parabiotic partner whose liver parenchyma had been damaged by carbon tetrachloride. It is clear, as has long been established, that in the partially hepatectomized liver there is an increase in mitosis. In order to decide whether the individual mitotic rates for the untreated partners of Group A, as compared with the partners of Group B, with carbon-tetrachloride-induced liver damage, were the product of our experimental methods or just coincidence, we subjected the data to statistical analysis.* From published reports²⁴ of other investigators and our own preliminary experiments, we know that mitosis in the liver of the white rat is very infrequent. This is shown again in Group C, which is, however, too small for statistical considerations. Brues and Marble²⁴ found that the mitosis in different equal-sized microscopic fields from the same rat followed the Poisson distribution.

If we were to assume that the number of mitoses in different pairs of animals follow the Poisson distribution, then the larger mitotic counts (for example, greater than nine) would be very infrequent. The possibility of a mitotic count equal to, or larger than, 10 would, using the example of Brues and Marble, appear in 1 out of 10^6 chances. This means that by counting the mitoses in 1,000,000 microscopic fields one would find, on the average, only one field with 10 or more mitoses.

Examining our results in Groups A and B, three of the eight undamaged partners of Group A showed mitotic rates of less than 10, and can, therefore, be considered normal. Consequently, 5 animals definitely show an increase in mitosis. In Group B, by the same criteria, only 3 of the 18 partners with liver damage show an increase in mitotic rate—in other words, of the 18 partners with preliminary carbon-tetrachloride-induced liver damage, only 3 animals showed

increases in mitotic rates, as opposed to 5 of the 8 undamaged partners of Group A.

Testing the results with the χ^2 -test, we found the difference to be significant ($\chi^2 = 5.244$; $P = < 0.05$). This significance was also confirmed by the more exact method of Fisher.²⁵

It was apparent from statistical calculations that the mitotic impulse, initiated from the partially hepatectomized partner, resulted in a significantly more frequent increase in mitosis in the undamaged partner than in the partner with carbon-tetrachloride-induced liver damage.

That the mitotic impulse is due to the partial hepatectomy and is transmitted to the parabiotic partner, is proved by the results of the control group (C), in which there was no partial hepatectomy. From this group, in which there was no increase in mitosis, we can eliminate as possible causes of the impulse (1) the parabiotic operation and its postoperative results, (2) the carbon-tetrachloride-induced liver damage, and (3) colchicine.

The fact that three of the eight undamaged animals did not respond to the mitotic impulse may be due to individual resistance or lack of transmission of the mitotic impulse because of incomplete parabiotic union.

Since we have established that the mitotic impulse is less frequently transmitted to a parabiotic partner with liver damage induced by carbon tetrachloride, it would seem logical to assume that the animals with the greatest damage from carbon tetrachloride which is demonstrable histologically would respond least to the mitotic impulse. While the number of animals in this study is too small to permit evaluation of any real correlation, it is worthy of note that in Pair 8 (Group B) there was no mitotic increase in spite of the insignificant liver damage and in Pair 13 (Group B) there was a definite increase in mitosis, despite a definite liver damage. One can therefore assume that there is some "blockade" of the mitotic impulse that is not discernible histologically.

* Statistical analysis directed by Mr. G. Güetli.

Summary

Using paired, parabiotically united white rats, we compared the effects of the mitotic impulse, induced by partial hepatectomy, on the healthy partner with those on the partner which had previously received carbon tetrachloride to induce liver damage.

The mitotic impulse which originates from the partially hepatectomized partner generally causes an increase in mitosis of the liver cells in the undamaged partner (62% of our animals). However, in the partner with liver damage induced by carbon tetrachloride the increase in mitosis is significantly less (17%). The ability of the damaged liver to react to the mitotic impulse is therefore diminished.

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REFERENCES

- Podwyszozi, W.: Experimentelle Untersuchungen über die Regeneration der Drüsengewebe; Untersuchungen über die Regeneration des Lebergewebes, Beitr. path. Anat. 1:254, 1886.
- Ponfick, E.: Experimentelle Beiträge zur Pathologie der Leber, Arch. path. Anat. 118:209, 1889.
- von Meister, V.: Recreation des Lebergewebes nach Abtragung ganzer Leberlappen, Beitr. path. Anat. 15:1, 1894.
- Higgins, G. M., and Anderson, R. M.: Experimental Pathology of the Liver: Restoration of the Liver of the White Rat Following Partial Surgical Removal, Arch. Path. 12:186, 1931.
- Lacquet, A. M.: Experimental Pathology of the Liver: VIII. Effects of Carbon Tetrachloride on the Normal and on the Restored Liver After Partial Hepatectomy, Arch. Path. 14:164, 1932.
- Novikoff, A. B., and Potter, V. R.: Biochemical Studies on Regenerating Liver, J. Biol. Chem. 173:223-232, 1948.
- Gurd, F. N., and Vars, H. M.: Pathologic Changes After Partial Hepatectomy with Special Reference to Hepatic Necrosis in Protein-Depleted Rats, Arch. Path. 48:140-149, 1949.
- Aterman, K.: Some Local Factors in the Restoration of the Rat's Liver After Partial Hepatectomy: I. Glycogen; the Golgi Apparatus; Sinusoidal Cells; the Basement Membrane of the Sinusoids, A.M.A. Arch. Path. 53:197-208; II. "Watery Vacuolation": Its Relation to the Vacuolation of Anoxia, *ibid.* 53:209-216, 1952.
- Glinos, A. D., and Gey, G. O.: Humoral Factors Involved in the Induction of Liver Regeneration in the Rat, Proc. Soc. Exper. Biol. & Med. 80:421-425, 1952.
- Norberg, B.: Alkaline Liver Phosphatases in Regenerating Rat Liver: Influence of Alloxan Diabetes, Insulin, and Bayer 205, Acta endocrinol. 11:156-170, 1952.
- Weinbren, K.: The Effect of Bile Duct Obstruction on Regeneration of the Rat's Liver, Brit. J. Exper. Path. 34:280-289, 1953.
- Doniach, I., and Weinbren, K.: The Development of Inclusion Bodies in the Cells of the Rat's Liver After Partial Hepatectomy, Brit. J. Exper. Path. 33:499-505, 1952.
- Sutherland, A. M.: Regeneration in the Fatty Liver of the Rat After Partial Hepatectomy, J. Path. & Bact. 71:403-408, 1956.
- Bucher, N. L. R.; Scott, J. F., and Aub, J. C.: Regeneration of the Liver in Parabiotic Rats, Cancer Res. 11:457-465, 1951.
- Dustin, A. P.: L'action des arsenicaux et de la colchicine sur la mitose la stathmocinèse, Compt. rend. Assoc. Anat. 33:205, 1938.
- Studer, A.: Zur Frage der Angriffsorte von Compound E (Cortison), Ztschr. ges. exper. Med. 121:287-418, 1953.
- Bunster, E., and Meyer, R. K.: An Improved Method of Parabiosis, Anat. Rec. 57:339, 1933.
- Sauerbruch, F., and Heyde, M. M.: Über Parabiose künstlich vereinigter Warmblüter, München. med. Wchnschr. 55:153, 1908.
- Bert, P.: Experiences et considerations sur la greffe animale, J. anat. et physiol. 1:64, 1864.
- Bickel, J., and Holm-Jensen: Determination by Means of Radioactive Blood Corpuscles of the Crossed Blood Flow Between Parabiotic Mice, Acta physiol. scandinav. 17:255, 1949.
- Van Dyke, D. C.; Huff, R. L., and Evans, H. M.: The Efficiency of the Vascular Union in Parabiosis, Stanford M. Bull. 6:271, 1948.
- Huff, R. L.; Trautman, R., and Van Dyke, D. C.: Nature of Exchange in Parabiotic Rats, Am. J. Physiol. 161:56, 1950.
- Ingle, D. J.: Technique of Repeated Partial Hepatectomy in the Rat, Proc. Soc. Exper. Biol. & Med. 87:251-253, 1954.
- Brues, A. M., and Marble, B. B.: An Analysis of Mitosis in Liver Restoration, J. Exper. Med. 65:15-27, 1937.
- Fisher, R. A.: The Exact Treatment of 2x2 Tables, from Statistical Methods for Research Workers, Ed. 8, Edinburgh and London, Oliver & Boyd, Ltd., 1941, p. 94.
- Hurowitz, R. B.: Thesis, Basle, Switzerland, to be published.

Oncocytoma (Oxyphil-Cell Adenoma) of the Caruncle of the Eyelid

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In 1897 Schaffer¹ first reported the occurrence of "granular swollen cells" in the ducts and acini of salivary glands of the tongue, uvula, pharynx, and esophagus. Hamperl² gave a complete description of these cells and called them "onkocytes" because of their large size, while McFarland^{3,4} referred to them as "Hürthle cells." Since these early reports several articles and excellent reviews⁵⁻⁷ have been published on the origin and pathogenesis of these cells. Although Hamperl⁷ states that the parotid gland is the commonest site, oncocytoma has been encountered in the submaxillary, sublingual, and minor salivary glands; in the thyroid, parotid, hypophysis, testis, Fallopian tube, pancreas, liver, and stomach, and the glands of the pharynx, trachea, and esophagus.

We encountered a case of oncocytoma of the caruncle of the eye. A review of literature has failed to reveal any published case of this lesion in this location, although correspondence⁸⁻¹⁰ indicates that two or three cases are known.

Report of a Case

A 63-year-old Mexican housewife came to the White Memorial Hospital and Clinic complaining of headache, poor vision in both eyes, burning sensation, and excessive lacrimation of the right eye. A small, reddish, elastic, smooth-surfaced growth was found extending out at the inner canthus of the right eye (Fig. 1). Ophthalmoscopic examination revealed no pathological findings. The right caruncle was excised completely, including

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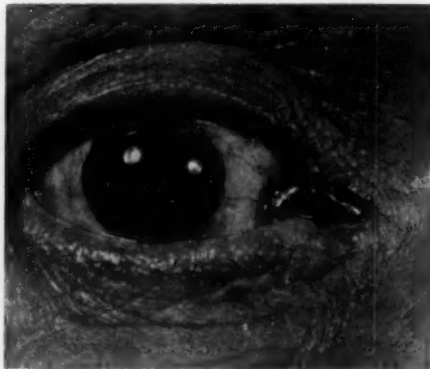


Fig. 1.—Oncocytoma (oxyphilic-cell adenoma) of right caruncle (Photo No. 38919). It is a small, pink, smooth-surfaced tumor, measuring $1 \times 0.5 \times 0.5$ cm. in size.

the plica semilunaris, on Dec. 22, 1957. There is no evidence of recurrence to the present time.

Pathological Examination.—On gross inspection, the tissue from the right caruncle appeared as a triangular grayish-white, smooth-surfaced, firm mass, measuring $1 \times 0.5 \times 0.5$ cm.

Microscopic section of tissue with routine hematoxylin-eosin staining showed a well-circumscribed, rounded tumor, composed of large oxyphilic cells in solid masses and forming small acini in some areas. The tumor was encapsulated and divided into lobules (Fig. 2) by thin connective tissue septa, which were infiltrated with numerous lymphocytes (Fig. 3). The epithelial cells were arranged in groups to form a lobular or tubular pattern. The cells were mostly large, spherical, oval, columnar, pyramidal, or polyhedral, with distinct boundaries. Some formed cords; others, acini or tubules. Cytoplasm was abundant and granular, staining intensely with eosin. The granules were small and regular in size. A fine reticulated structure was made out in the cyto-



Fig. 2.—Oncocytoma (oxyphilic-cell adenoma) of right caruncle (entire view). It is a well-circumscribed, smooth-surfaced, lobulated tumor mass containing tumor nodules and dilated ducts. $\times 14$.

plasm of some cells, and the granules lay in the interstices. The nuclei were small, round, or oval, with evenly distributed, fine granular chromatin (Fig. 4).

There were numerous serous secretory ducts surrounding these masses of cells. Many of the ducts were dilated and contained pink-staining amorphous material.

The tumor cells strikingly resembled those of the ducts. There was no evidence of malignancy.

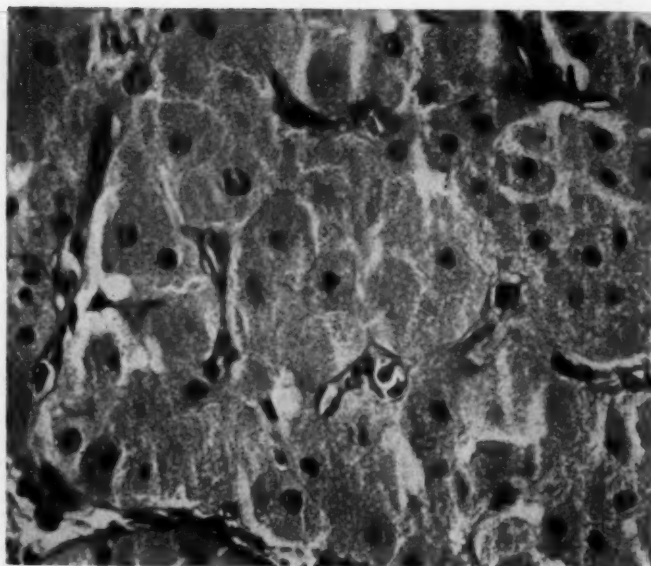
Comment

The exact origin of the oncoocyte and oncocytoma is not known, but McFarland^{3,4} believed that these oxyphilic granular cells

Fig. 3.—Oncocytoma (oxyphilic-cell adenoma) of right caruncle (Photo No. 39665), showing groups of oxyphilic cells separated by delicate fibrous bands. The ducts are markedly dilated, containing pink-staining amorphous material. Reduced to 95% of mag. $\times 100$.



Fig. 4.—Oncocytoma (oxyphilic-cell adenoma) of right caruncle (Photo No. 39717). The cells are large, round, oval, or polyhedral. The cytoplasm is abundant, granular, and stained intensively with eosin. The nuclei are small, round, or oval, with fine granular chromatin, evenly distributed. Reduced to 95% of mag. $\times 450$.



arise from the gland by transformation of its cells into a new type, giving an impression of neoplastic transformation rather than hyperplasia. Recurrences following attempted removal of these tumors have been reported.^{3,4,11-13} Gruenfeld and Jorstad¹⁴ and Harris¹⁵ stated that the tumor may originate from ducts. Meza-Chavez¹⁶ traced the origin of the oncocytoma cells of the parotid gland to both ducts and acini which had undergone transformation and attempted to explain the origin of the tumor by a possible connection with the sebaceous gland. Stump¹⁷ suspected that oncocytomas represented a unique form of retrogressive cellular alteration, inasmuch as they are present in the aged but not in adolescents.

Oncocytomas have usually been described as unicellular tumors with the exception of the ones reported by Christopherson¹⁸ and Foote.¹⁹ In these two instances, small foci with many structural characteristics of mixed tumors were noted. Schafer et al.²⁰ stated that tumors containing mesenchymal elements are actually mixed tumors which have undergone oncocytic transformations.

The histogenesis of the oncocytoma in the caruncle of the eye has not clearly been

demonstrated. The caruncle contains sweat glands, and occasionally abortive lacrimal glands,²¹ which may be placed there by displacement during their development. The lacrimal glands develop during the ninth week of embryonic life, arising from the conjunctival epithelium of the lateral part of the upper lid in the area, where it turns back to be reflected over the sclera.²² It is not inconceivable that the ducts from these glands may give rise to the oncocytoma. Cases of so-called adenoma of the lacrimal gland under the conjunctiva has been reported, but these are actually abortive lacrimal glands rather than tumors.²³ No true adenoma of the lacrimal gland has been encountered. It is also possible that the oncocytoma may arise from the epithelium of the sweat glands or by metaplasia of the myoepithelioid elements.

Summary

An oncocytoma (oxyphilic-cell adenoma) of the caruncle of the eye in a 63-year-old Mexican woman is reported.

Review of the literature has failed to reveal any reported cases of such lesion.

The oncocytoma may have arisen from (a) abortive lacrimal glands or (b) the epithelium of the sweat glands present in the caruncle.

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REFERENCES

1. Schaffer, J.: Beiträge zur Histologie menschlicher Organe, Sitzungsber. k. Akad. Wissensch. Math.-naturw. Cl. 106 (Pt. 3):353-455, 1897.
2. Hamperl, H.: Beiträge zur normalen und pathologischen Histologie menschlicher Speicheldrüsen, Ztschr. f. mikr.-anat. Forsch. 27:1-55, 1931.
3. McFarland, J.: Ninety Tumors of the Parotid Region in All of Which the Postoperative History Was Traced, Am. J.M. Sc. 172:804-848, 1926.
4. McFarland, J.: The Mysterious Mixed Tumors of the Salivary Glands, Surg. Gynec. & Obst. 76:23-34, 1943.
5. Meza-Chavez, L.: Oxyphilic Granular Cell Adenoma of the Parotid Gland (Oncocytoma): Report of 5 Cases and Study of Oxyphilic Granular Cell (Oncocytes) in Normal Parotid Glands, Am. J. Path. 25:523-538, 1949.
6. Greenberg, S. D., and Haley, M. D.: Oncocytoma (Oxyphil Cell Adenoma) of the Parotid Gland, Am. J. Clin. Path. 27:321-327, 1957.
7. Hamperl, H.: Über das Vorkommen von Onkocyten in verschiedenen Organen und ihren Geschwülsten, Virchows Arch. path. Anat. 298:325-375, 1936-1937.
8. Reese, A. B.: Personal correspondence, 1958.
9. Perkhill, E.: Personal correspondence, 1958.
10. Hamperl, H.: Personal correspondence, 1958.
11. McFarland, J.: Adenoma of the Salivary Glands, with a Report of a Possible Case, Am. J.M. Sc. 174:362-378, 1927.
12. McFarland, J.: Three Hundred Mixed Tumors of the Salivary Glands of Which 69 Recurred, Surg. Gynec. & Obst. 63:457-468, 1936.
13. Ackerman, L. V.: Oncocytoma of the Parotid Gland, Arch. Path. 36:508-511, 1943.
14. Gruenfeld, G. E., and Jorstad, L. H.: Adenoma of the Parotid Salivary Gland: Oncocyte Tumor, Am. J. Cancer 26:571-575, 1936.
15. Harris, P. N.: Adenoma of the Salivary Glands, Am. J. Cancer 27:690-700, 1936.
16. Meza-Chavez, L.: Sebaceous Glands in Normal and Neoplastic Parotid Glands: Possible Significance of Sebaceous Glands in Respect to the Origin of Tumors of the Salivary Glands, Am. J. Path. 25:627-645, 1949.
17. Stump, D. J.: Onkocytic Adenoma of the Salivary Gland, Arch. Path. 48:287-296, 1949.
18. Christopherson, W. M.: Oncocytoma of the Parotid Gland, Arch. Path. 48:96-98, 1949.
19. Foote, R. W., Jr., and Frazell, E. L.: Tumors of the Major Salivary Glands, in Atlas of Tumor Pathology, Sec. IV, Fasc. 2, Armed Forces Institute of Pathology, National Research Council, Washington, D.C., 1954, pp. 137-144.
20. Schafer, E. L.; Gruet, M., and Jackson, A. S.: Oncocytic Cell Adenoma of the Parotid Gland, Am. J. Surg. 91:273-278, 1956.
21. Maximow, A. A., and Bloom, W.: Textbook of Histology, Ed. 7, Philadelphia and London, W. B. Saunders Company 1957, p. 579.
22. Patten, B. M.: Human Embryology, Ed. 2, New York, The Blakiston Company (Division of McGraw-Hill Book Company, Inc.), 1953.
23. Reese, A. B.: Tumor of the Eye and Adnexa, in Atlas of Tumor Pathology, Fasc. 38, Armed Forces Institute of Pathology, National Research Council, Washington, D.C., 1956, p. 190.

Pathology of the Ganglionic-Aganglionic Junction in Congenital Megacolon

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Defective autonomic nerve supply of the bowel wall is accepted generally as the principal anatomical cause of congenital megacolon (Hirschsprung's disease)¹⁻³ and abnormal peristalsis as the functional effect.⁴ This combination produces narrowing with obstruction in the affected segment and dilatation proximally. Numerous reviews have summarized the pathology and have described the clinical and radiological manifestations⁵⁻⁷ of the common case and rarer variants. Successful surgical therapy, consisting of removal of the aganglionic segment of bowel, has been based on this foundation.⁸ Biopsy has aided diagnosis by confirming a presumed aganglionosis and has refined therapy by fixing the limits of effective resection.^{9,10}

Recent reports^{11,12} have been aimed at descriptions of the extent of longitudinal involvement of bowel, whereas a comparison of the histology of the nerve supply in various layers at the junction of ganglionic and aganglionic bowel has not been made.

The present study has been designed to clarify the question of whether there is correspondence between the terminations of ganglion cells in Meissner's (submucosal) and Auerbach's (myenteric, intermuscular) plexuses in Hirschsprung's disease. Decisions regarding the depth or extent of biopsy needed for effective results depend on familiarity with such relationships.

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Materials and Methods

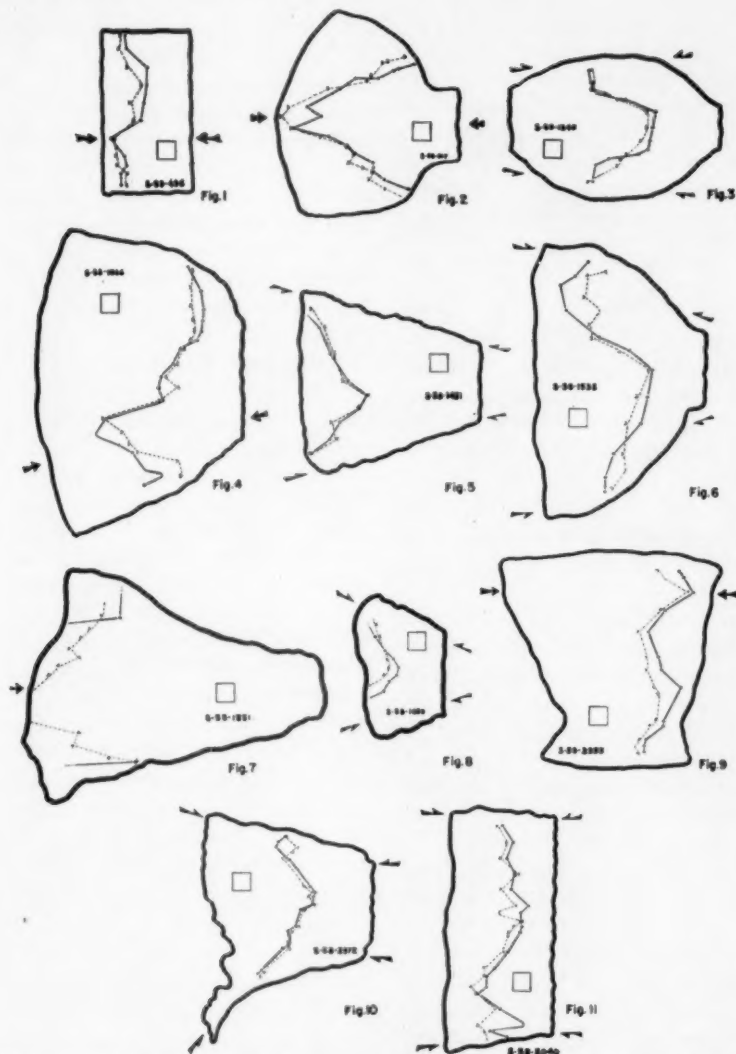
Eleven specimens of large bowel resected from cases of Hirschsprung's disease were carefully mapped out to demonstrate the location of the most caudal ganglion cells of both Auerbach's and Meissner's plexus. Only those cases were used in which a significant portion of the, or the entire, junction between ganglionic and aganglionic bowel was uninterrupted by surgical intervention and was contained within the main resected specimen. Otherwise, the cases were unselected and were studied in sequence.

The specimens were opened longitudinally along either the mesocolic or the antimesenteric line and fixed flat in 10% formalin. The approximate location of the ganglionic margin was determined by pilot samplings and was subsequently defined by semiserial longitudinal blocks taken close together, largely in parallel, along the entire circumference.

Special care was taken to include clearly the ganglion-cell termination in both plexuses within each block. The end-points were checked wherever possible in adjacent serial sections and were recorded graphically to a scale of 1:1. Only those neurons were recorded which were clearly identifiable as such, principally by their prominent nucleolus, but also by other well-established nuclear and cytoplasmic characteristics.

Observations

The change from a normally ganglionic autonomic nerve supply to totally aganglionic bowel was not completely abrupt in all cases. The presence of a zone where neurons are scanty was confirmed in some instances, but its extent and character were not evaluated. The end-points generally were fairly sharp and could be substantiated in adjacent serial microscopic sections. Such a gradual diminution of neurons, with stragglers so widely spaced as to make the level of termination indistinct, was rarely encountered.



Figs. 1-11.—Outline drawings of longitudinally opened segments of large bowel in Cases 1-11, all oriented so that the proximal margin of resection is on the left and the distal margin on the right. The most distal ganglion cells identified in the myenteric plexus are designated by dots, connected by unbroken heavy lines. The most distal ganglion cells identified in the submucosal plexus are designated by checks (v) connected by light broken lines. The arrows indicate the median line of mesenteric attachment. The small square for each specimen stands for a square centimeter.

The junction in all cases is delineated at both submucosal and intermuscular levels in line drawings (Figs. 1-11). Three main observations may be made from these.

1. The lines representing the termination of ganglion cells in the submucosal and intermuscular plexuses generally coincide.

2. The junction tends to be more caudally located opposite to the line of mesenteric attachment. Cases 2, 3, 4, 5, 6, 7, 8, and 10 bear this out clearly.

3. The submucosal ganglion cells appear to terminate either at the same level or a short distance cephalad to the intermuscular

ganglion cells. This trend is particularly noticeable in the antimesenteric portion of the bowel wall, as in Cases 1, 2, 3, 6, and 8. Minor exceptions, where the submucosal ganglion cells can be traced more distally than their intermuscular counterparts, can be found in almost every case. The discrepancy is minimal or absent in some instances (Figs. 5 and 10).

Comment

It would seem clear that the general correspondence of the junction at the submucosal and intermuscular levels has been conclusively demonstrated. The more distal extent of the ganglionic margin along the antimesenteric line and the "lag" between submucosal and intermuscular end-points appear to be of less significance, more variable, and subject to differences of interpretation.

One factor which may play an important role in bringing about the more extensive antimesenteric ganglioneosis may be a mechanical one of greater dilatation opposite to the point of attachment of the bowel. In many cases the longest convexity relative to the mesocolic point of attachment lies longitudinally along the antimesenteric line. This bulge effect may produce a distribution of the neuronal units over a larger area, thereby bringing about a relative and acquired level difference rather than an absolute and developmental one. This finding cannot be ascribed to artifact of sectioning and fixation, for it is demonstrable irrespective of the line along which the bowel was opened. It is also more noticeable where dilatation is most pronounced, as indicated by the configuration of the specimens, e.g., Cases 2, 3, 4, 5, 6, 7, 8, and 10.

The findings of a minor and inconstant longitudinal "lag" between end-points must be regarded in the light of several considerations. First, the problem of fixation shrinkage becomes important: Such an effect tends to minimize these longitudinal end-point differences and to distort them where a greater contraction of muscle lay-

ers with respect to submucosa makes shrinkage a differential one.

A more random distribution of ganglionic elements in a greater volume of loose areolar tissue of the submucosa may make localization of these elements less precise than in the intermuscular layer, where they are contained in a much narrower space. The latter offers a distinct advantage for rapidity and accuracy of localization. This is particularly true when immediate diagnosis is required.

Lastly, this discrepancy may be considered on a theoretical basis with respect to the development and caudal displacement of the hindgut relative to a more cephalic source of autonomic nerve supply. In pig embryos neuronal cells accompany nerve fiber components from the pelvic plexus into the wall of the hindgut and apparently become incorporated into the enteric ganglia.¹⁰ The appearance of ganglion cells is dependent on the penetration of these nerve fiber components. If one assumes that the ultimate position of ganglion cells in their relatively caudad migration is a linear function of the nerve fibers which join them, then any diminution in the longitudinal distance of their position from a fixed point of origin may be ascribed to lateral or transverse deflection. In the penetration of the inner circular muscle layer, such a loss, though small, might be incurred and could explain a more cephalic position of submucosal neuronal elements relative to their intermuscular counterparts.

Turning to a more practical consideration, it is clear that the absence of ganglion cells is the most reliable single diagnostic criterion in congenital megacolon, or Hirschsprung's disease. Scantiness of neurons, poor formation, their gradual diminution in the transitional segment are highly subjective and unreliable criteria. Similarly, any increase in number, or in size or complexity of nonmyelinated nerve fibers in the aganglionic portion is an inconstant finding, difficult to evaluate. On the contrary, the two muscle layers, distal to the ganglionic

GANGLIONIC-AGANGLIONIC JUNCTION

margin, are frequently tightly apposed, with few intervening nerve fiber bundles.

In the employment of rectal biopsy, the choice between mucosal and submucosal biopsy and whole-thickness or muscle biopsy must be governed by several anatomical considerations. The general correspondence of ganglionosis in the two plexuses and the fact that a diagnostic biopsy specimen is generally taken in the rectum, far beyond the ganglionic-aganglionic junction, would indicate that a submucosal biopsy might suffice. Such a choice must be tempered by the realization that a positive diagnosis of Hirschsprung's disease, on which therapeutic surgical intervention is based, depends on a negative finding. This generally takes place at an age when ganglion cells, even if present, may be few, inconspicuous, small, or poorly formed, and are identified more easily in the plexus of Auerbach. Substitution of surface sampling for full-thickness biopsy in the interest of speed and simplicity must be based on proper appraisal of the risk of diagnostic inaccuracy against the risks of possible postbiopsy complications, and should be avoided whenever possible.

Summary

The ganglionic-aganglionic junction in the autonomic plexuses of the colon in Hirschsprung's disease was studied. A general correspondence of level and configuration of the ganglionic margin was found between the submucosal plexus of Meissner and the intermuscular plexus of Auerbach. Minor details of variation were noted. The findings are discussed with regard to embryogenesis and to practical application in diagnostic biopsy.

I am indebted to Drs. Orvar Swenson and J. Herbert Fisher, from whose services this material was obtained, and to Mrs. E. Langille for her technical assistance.

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REFERENCES

1. Tittel, K.: Über eine angeborene Missbildung des Dickdarmes, 14:903-907, 1901.
2. Dalla Valle, A.: Contributo alla conoscenza della forma familiare del megacolon congenito, *Pediatrics* 32:569-599, 1924.
3. Robertson, H. E., and Kernohan, J. W.: The Myenteric Plexus in Congenital Megacolon, *Proc. Staff Meet. Mayo Clin.* 13:123-125, 1938.
4. Swenson, O.; Rheinlander, H. F., and Diamond, I.: Hirschsprung's Disease: A New Concept of the Etiology: Operative Results in 34 Patients, *New England J. Med.* 241:551-556, 1949.
5. Bodian, M.; Carter, C. O., and Ward, B. C. H.: Hirschsprung's Disease, *Lancet* 1:302-309, 1951.
6. Bodian, M.; Stephens, F. D., and Ward, B. C. H.: Hirschsprung's Disease and Idiopathic Megacolon, *Lancet* 1:6-11, 1949.
7. Swenson, O.: Hirschsprung's Disease (Aganglionic Megacolon), *New England J. Med.* 260:972-976, 1959.
8. Swenson, O.: Modern Treatment of Hirschsprung's Disease, *J.A.M.A.* 154:651-653, 1954.
9. Swenson, O.; Fischer, J. H., and MacMahon, H. E.: Rectal Biopsy as an Aid in the Diagnosis of Hirschsprung's Disease, *New England J. Med.* 253:632-635, 1955.
10. Swenson, O.; Fischer, J. H., and Gherardi, G. J.: Rectal Biopsy in the Diagnosis of Hirschsprung's Disease, *Surgery* 45:690-695, 1959.
11. Riker, W. L.: Diagnosis and Therapy of Aganglionosis of the Myenteric Plexus, *A.M.A. Arch. Surg.* 75:362-375, 1957.
12. Boggs, J. D., and Kidd, J. M.: Congenital Abnormalities of Intestinal Innervation: Absence of Innervation of Jejunum, Ileum and Colon in Siblings, *Pediatrics* 21:261-266, 1958.
13. Kuntz, A.: Origin and Early Development of Pelvic Neural Plexuses, *J. Comp. Neurol.* 96:345-357, 1952.

Vascular Changes Induced by Bacterial Endotoxin During Generalized Schwartzman Reaction

Effect of Cortisone

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With the Technical Assistance of Marcia Oberlander

Alterations in the circulation of blood through the liver, lungs, spleen, and kidney during the course of the generalized Schwartzman reaction have recently been described by McKay and Rowe.^{1,2} This study was based on the distribution of India ink injected into the aorta at various times after the injection of one and two doses of bacterial endotoxin. Macroscopic and microscopic changes were observed. One hour after the first injection of endotoxin, large "ischemic" areas appeared in the lungs. These were caused by arterial platelet thrombi, and possibly by arterial vasospasm. Alternating with these areas were "blackened" patches in which the alveolar capillaries were dilated and had trapped large amounts of ink. Similar, but less distinct, areas appeared in the spleen and liver, and these were also associated with arterial platelet thrombi. Four hours after each injection of endotoxin there appeared a virtually complete exclusion of ink from the center of all hepatic lobules. This obstruction to blood flow in the liver was associated with a dilation of the peripheral sinusoids of the hepatic lobules. The renal

glomeruli contained a few India ink granules during all stages of the Schwartzman reaction, until two to four hours after the second injection. At this time the glomerular capillaries dilated and filled with ink. This stasis of blood in these vessels preceded or was concomitant with the intravascular deposition of fibrin in the glomerular capillaries.

It was suggested that dilation and stasis of blood in the renal glomeruli might be the conditioning factor in the subsequent localization of thrombi in these vessels and therefore might constitute one of the major mechanisms of "preparation" in the generalized Schwartzman reaction. To test this idea further, the effects of cortisone on the vascular pattern before and after endotoxin injection were studied in a similar manner. The experiment differs only in replacing the first injection of endotoxin by cortisone administration. In this instance, according to Thomas and Good,³ only one dose of endotoxin is needed to induce the renal thrombi of the generalized Schwartzman reaction. If glomerular dilation and stasis constitute an important mechanism in causing the thrombi to localize in these capillaries, these changes should appear after but one injection in animals prepared with cortisone. In addition to the renal vascular response, changes were observed in the small vessels in other organs which were different from those in the previous experiments, and are reported herein.

Materials and Methods

Hybrid female albino rabbits, weighing approximately 1 kg. and fed on Purina Rabbit Chow, were

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From the Pathology Research Laboratory of the Free Hospital for Women, Brookline, Mass. and the Departments of Pathology, Obstetrics, and Gynecology, Harvard Medical School, Boston.

given 25 mg. of cortisone intramuscularly for three successive days. For the injection of India ink into the aorta they were anesthetized with pentobarbital sodium. Procaine was injected locally; the femoral artery was freed up, and a polyethylene catheter was inserted as soon as the animal was anesthetized. The catheter was inserted so that the tip lay in the aorta at the level of the diaphragm. At this point 10 ml. of a 10% suspension of Higgins India ink in isotonic saline was injected into the aorta by means of a glass syringe. A few animals received the injection by way of the left ventricle. The injection was accomplished over a period of two to three minutes. The abdomen was opened immediately after injection of India ink, and the lungs, spleen, liver, adrenals, and kidneys were removed. The animal's death occurred as the result of hemorrhagic shock consequent to the removal of these organs. After placing sections of these organs in formalin, a complete autopsy was done. The blocks were prepared in the standard way and embedded in paraffin. Six-micron sections were cut and stained with hematoxylin and eosin and phosphotungstic acid hematoxylin. Thirty-micron sections were cut and mounted unstained on glass slides.

In this study there are actually three control groups and three experimental groups.

Control Group I.—This group was comprised of animals given two doses of toxin and injected with India ink at 0, 1, 2, 4, and 24 hours after the first and second injections. These have been previously reported.^{1,2}

Control Group II.—This group, of eight animals, was pretreated with cortisone and then injected with 0.2 mg. of endotoxin and killed at 24 hours without the injection of India ink. The purpose of this control was to determine the effectiveness of endotoxin in producing the Schwartzman reaction and also to determine whether or not the pathologic changes after the cortisone "preparation" were the same as those following the endotoxin "preparation."

Control Group III.—This group, of four animals, received cortisone pretreatment and were injected with India ink but not endotoxin. The purpose of this control was to determine the effect of cortisone alone on the vascular pattern.

Experimental Groups.—Each of these three groups were pretreated with cortisone and then given 0.2 mg. of endotoxin. In Group A, four animals were injected with India ink one hour after endotoxin; in Group B, four animals were injected with ink two hours after endotoxin, and in Group C, eight animals were injected with ink four hours after endotoxin. These last three groups contain the actual experiments of this study.

McKay—Merriam

Observations

For the purposes of orientation, in describing the observations, we have presented the pathologic findings (Control Group II) first. Control Group III, which demonstrates the effects of cortisone alone on the distribution of India ink, is presented next. Comparisons with the two injection experiments (Control Group I) are made at the points where differences exist. The second section of this description includes the experimental Groups A, B, and C, and they are described together according to organ involvement.

Control Group II.—In the eight animals examined four hours after receiving endotoxin, glomerular capillary thrombi were found in five. Of the animals examined 24 hours after endotoxin, bilateral renal cortical necrosis occurred in two, and two others had glomerular capillary thrombi.

With respect to the kidneys, spleen, and liver, the lodgment of thrombi in small vessels, followed by the development of hemorrhage or necrosis in the peripheral tissue was essentially the same as that seen after the second injection of toxin in the standard two-dose Schwartzman reaction.^{1,2} The liver, on the other hand, reacted quite differently. No thrombi were observed in the liver of any animals killed four hours or less after endotoxin injection. Only one animal out of eight sacrificed twenty-four hours after endotoxin showed thrombi and necrosis. This is in contrast with the standard Schwartzman reaction, in which hepatic thrombi were found in 9 of 37 animals given one dose of toxin and in 31 out of 45 animals given two doses of toxin.⁴ There were a few animals with focal necrosis which was interpreted as due to coccidiosis.

Increased numbers of megakaryocytes were observed in the lungs in the cortisone-treated animals. The numbers of these cells appeared to decrease 4 hours after endotoxin injection, but they were found in large numbers again 24 hours after exposure to endotoxin.

Control Group III.—From the macroscopic standpoint, the ink was distributed very much as in the control group of our previous experiments (Control Group I). The lungs appeared slightly gray, as did the kidneys, while the liver and spleen were diffusely blackened. Petechial hemorrhages were observed in the majority of these lungs.

Microscopically the pulmonary alveolar capillaries were packed full of ink. This was in contrast to control animals which were not pretreated with cortisone (i.e., normal animals), which had very little ink in the pulmonary capillaries. The distribution of ink in the cortisone-treated controls was actually very much like that in the animals which had received one dose of endotoxin in the previous studies (Control Group I).

Cortisone treatment caused a profound change in the histology of the liver. In the cortisone control group (III), as well as the others, the liver cells were swollen and had a clear cytoplasm, giving them the appearance of glycogen infiltration. The India ink was distributed evenly through every sinusoid but with slight tendency in a few lobules to "ischemia" of the central portion of the lobule. The spleens appeared no different from those of the previous study. The sinuses of the red pulp contained large, clumped masses of ink, and there was a concentration in the peripheral portion of each Malpighian corpuscle, while the central portion was completely devoid of ink.

The kidneys of this control group contained slightly more ink than the kidneys of the control group that did not receive cortisone. The adrenals were completely free of ink.

Experimental Groups A, B, and C (Endotoxin-Treated Animals).—A. Lungs: One hour after endotoxin injection the macroscopic appearance of the lungs was changed markedly. Large pink areas, completely free of ink alternated, with dense black areas in a geographic distribution (Fig. 1). Small hemorrhages were found in most lungs. This patchy distribution of ink oc-

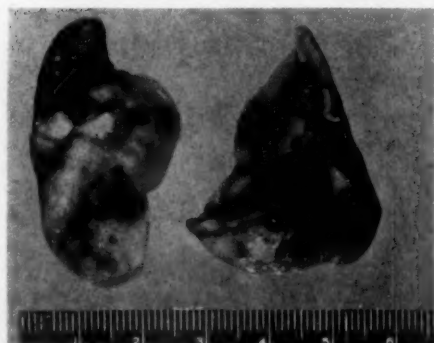


Fig. 1.—India ink-injected lungs, one hour after endotoxin. The pale areas are devoid of ink, and the blackened areas contain large amounts. Control animals show a diffuse, homogeneous gray lung surface. The alterations shown above persist throughout the experiment.

curred in all animals in the one, two and four-hour time periods. Microscopically, the pale areas were devoid of ink, and the alveolar capillaries of the black areas were filled with ink (Fig. 2).

B. Liver: The liver cells of all these animals were swollen and vacuolated diffusely (Fig. 3). The even, regular distribution of ink in every sinusoid observed in the control group was found in every animal following endotoxin injection. This was quite in contrast to the previous study (Control Group I), in which an ischemia of the centers of the lobules, accompanied by a dilatation of the peripheral sinusoids, occurred four hours after each injection of endotoxin.

C. Adrenals: At two and four hours after endotoxin injection the sinusoids of the adrenal contained appreciable amounts of ink. This amount of ink in the adrenal was not observed in the control animals of this series.

D. Pituitary: Most of the pituitary glands showed an increased amount of ink in the sinusoids four hours after endotoxin.

E. Kidneys: The macroscopic appearance of the kidneys did not change throughout the 48-hour period of study. There was a light-gray tinge to the otherwise reddish-brown renal cortex in all specimens.

Microscopically, the normal distribution of ink in sparse granules in the glomeruli

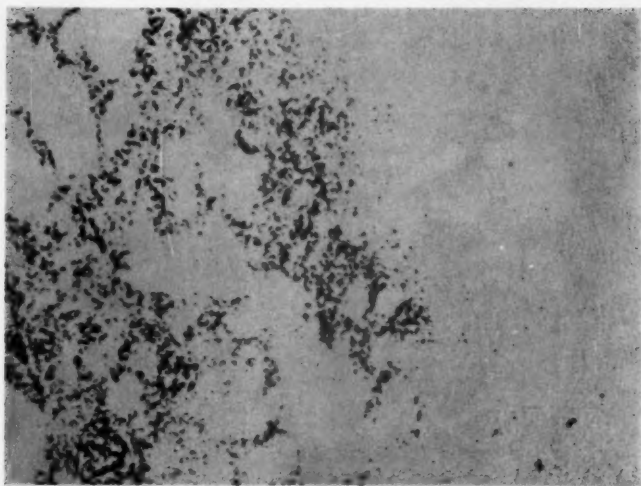


Fig. 2.—Lung one hour after endotoxin. This photomicrograph shows the dilated ink-filled alveolar capillaries on the left (one of the grossly blackened areas) and a patch on the right which is completely free of ink. Unstained section; 30 μ thick; $\times 100$.

and a few of the intertubular capillaries occurred in all of the kidneys one hour after endotoxin injection (Fig. 4). At two hours a slight increase in the amount of ink in the glomeruli appeared. A few glomeruli in three of these animals were heavily filled with ink. At four hours five out of eight animals showed a marked dilation of glomerular capillaries (Figs. 5 and 6). Two of the animals that did not show the glomerular dilation were mishandled. During the catheterization of the femoral artery a great deal of blood was lost, and the animals

were in shock. It is possible that they should be excluded from the final results, since a greatly decreased blood volume might be an adequate reason for a failure of the blood to flow through the kidney cortex, thus excluding India ink from the glomeruli for an extraneous reason.

Interpretation

Studies in the past have demonstrated that one of the fundamental features of the pathogenesis of the generalized Schwartzman

Fig. 3.—Liver of control animal. These vacuolated liver cells were found in all the cortisone-treated animals whether or not they received endotoxin. The pattern of distribution of ink was also the same in all these livers. Four hours after endotoxin there was no change in the regular, diffuse distribution of ink in the sinusoids. This is in contrast to the standard two-dose Schwartzman reaction, in which four hours after each endotoxin injection there was an absence of ink from the central portion of the lobules and a dilatation of the peripheral sinusoids. Hematoxylin-eosin stain; 6 μ thickness; $\times 200$.

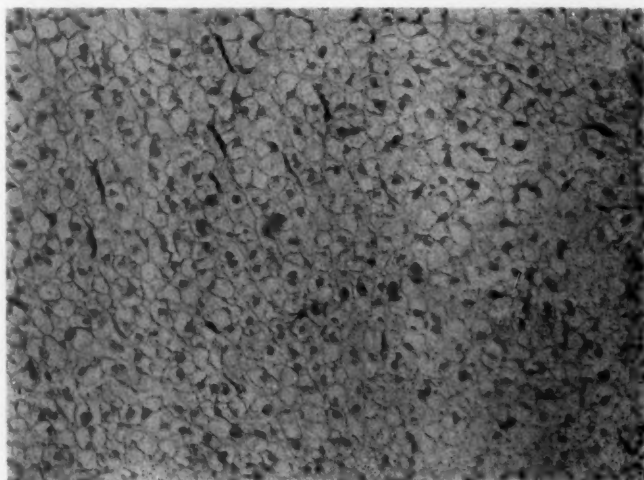


Fig. 4.—Kidney, control animal. A few granules of ink are present in the capillaries of the kidney. Unstained section; 30 μ thickness; $\times 100$.



reaction is the participation of the blood-coagulation system. The reaction may be prevented by blocking the coagulation of blood *in vivo* by heparin,⁵ warfarin sodium,⁶ and fibrinolytic activity.⁷ It is to be noted that intravascular coagulation occurs after both injections of endotoxin. In essence, the classical generalized Schwartzman reaction can be characterized as two episodes of disseminated intravascular coagulation. The peak response of the blood coagulation system to endotoxin is four hours after

each injection, and the fibrin thrombi appear between two and four hours. However, the first injection produces thrombi in the liver, lungs, and spleen, whereas the second injection causes them to appear in the kidney glomeruli. It is clear that some change has taken place after the first injection to "prepare" the kidney.

To determine the cause for the localization of fibrin in the glomeruli only after the second injection is the basic aim of our study. The previous experiments have raised

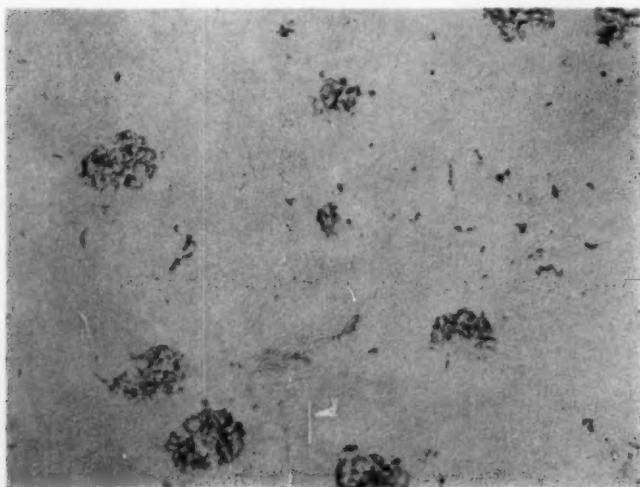


Fig. 5.—Kidney. Four hours after endotoxin the majority of glomeruli are dilated and filled with ink. Unstained section; 30 μ thick; $\times 100$.

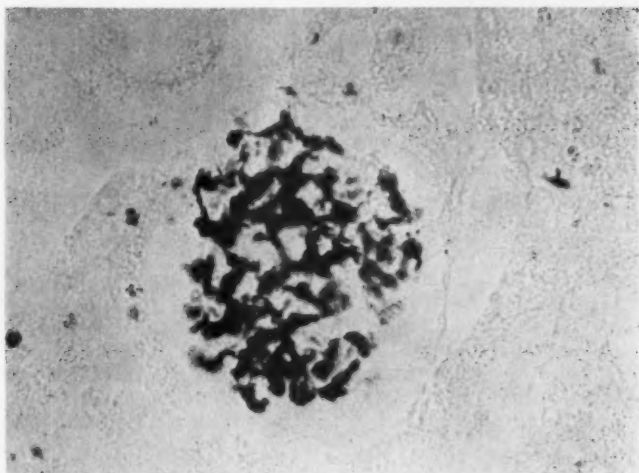


Fig. 6.—Kidney, glomerulus. Four hours after endotoxin. Unstained section; 30μ thick; $\times 400$.

the possibility that dilation of glomerular capillaries might be a basic factor, because the dilation or stasis appeared simultaneously and exclusively at the time of the appearance of the glomerular thrombi, two to four hours after the second injection of endotoxin. The present experiment demonstrates that the glomerular dilation and stasis occur after the first dose of endotoxin (i.e., the "provoking" dose) in animals prepared with cortisone, simultaneously and exclusively, at the time of the appearance of the glomerular thrombi. These observations support the concept that stasis of blood in the glomerular capillaries is an important factor in causing the localization of fibrin thrombi in the kidney, when disseminated intravascular coagulation is produced by bacterial endotoxin. Stated differently, glomerular capillary dilation appears to be a major element of "preparation" for the generalized Schwartzman reaction. The vascular system of other organs in the cortisone-prepared animals responded differently than in normal animals.

Cortisone preparation altered the pulmonary vascular pattern. The pulmonary alveolar capillaries dilated in response to the cortisone administration, whereas in the previous experiments these capillaries were not dilated in normal animals and became dilated only after injection of bacterial endotoxin.

No change was observed in the vascular pattern of the liver four hours after endotoxin in the cortisone-prepared animals. This is quite in contrast with the response of normal animals to endotoxin, in which a dilation of sinusoids at the periphery of each lobule with a centrolobular "ischemia" appeared four hours after each injection of endotoxin. It is noteworthy that in the cortisone-treated group all the liver cells were filled with glycogen and were greatly swollen. It may be that the enlargement of the liver cells was responsible for this failure of the sinusoids to respond to endotoxin in this experiment. On the other hand, it may be that the cortisone had a primary action on the endothelium or some other vascular structure of the liver to prevent the reaction. Whatever the mechanism, it is important to note that the absence of a vasomotor response in the liver was associated with a lack of thrombosis in this organ. In the standard Schwartzman reaction the converse was also true—namely, when vasomotor changes in the liver lobule occurred, thrombi were found in this location. In a general way it appears that in two locations, the liver and the kidney, thrombosis is associated with vasomotor activity (dilation of small vessels) and that in the absence of the dilation thrombosis tends not to occur.

Summary

Rabbits were prepared for the generalized Shwartzman reaction by cortisone pretreatment. They were then given an intra-aortic injection of India ink and examined histologically for alterations in the distribution of ink in the small vessels of the liver, lungs, spleen, kidney, adrenals, and pituitary. Although basically the same changes were observed in the redistribution of ink as had been seen in the standard two-dose generalized Shwartzman reaction, certain important differences were noted.

1. The renal glomerular capillaries dilated and filled with ink four hours after one injection of bacterial endotoxin. This was accompanied by glomerular capillary thrombosis.

2. No change was observed in the distribution of ink in the liver. Thrombosis of the hepatic sinusoids and central veins occurred in only 1 out of 16 animals.

Thus, in two organs, the liver and kidney, thrombosis of small vessels is closely related to an accompanying vascular dilation. When the dilation occurs, thrombi are found, and, conversely, when the dilation does not occur no thrombi are found. It is suggested that capillary dilation is an important factor in the localization of thrombi in the generalized Shwartzman reaction and may be considered as an important facet of the

phenomenon of "preparation" for this reaction.

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REFERENCES

1. McKay, D. G., and Rowe, F. J.: Effect of Bacterial Endotoxin on Small Blood Vessels During the Generalized Shwartzman Reaction, *Fed. Proc.* 18:493, 1959, abstract 1944.
2. McKay, D. G., and Rowe, F. J.: Effect of Bacterial Endotoxin on the Arterial Vascular System in the Generalized Shwartzman Reaction, *Lab. Invest.*, to be published.
3. Thomas, L., and Good, R. A.: Effect of Cortisone on the Shwartzman Reaction: Production of Lesions Resembling the Dermal and Generalized Shwartzman Reactions by a Single Injection of Bacterial Endotoxin in Cortisone-Treated Rabbits, *J. Exper. Med.* 95:409, 1952.
4. McKay, D. G., and Shapiro, S. S.: Alterations in the Blood Coagulation System Induced by Bacterial Endotoxin: I. In Vivo (Generalized Shwartzman Reaction), *J. Exper. Med.* 107:353-367, 1958.
5. Good, R. A., and Thomas, L.: Studies on the Generalized Shwartzman Reaction: IV. Prevention of the Local and Generalized Shwartzman Reaction with Heparin, *J. Exper. Med.* 97:871, 1953.
6. Shapiro, S. S., and McKay, D. G.: Prevention of the Generalized Shwartzman Reaction with Sodium Warfarin, *J. Exper. Med.* 107:377-381, 1958.
7. Kliman, A., and McKay, D. G.: The Prevention of the Generalized Shwartzman Reaction by Fibrinolytic Activity, *A.M.A. Arch. Path.* 66: 715-719, 1958.

Mast Cell Content of Placental Tissue

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Villi of placentas have been described as having a small number of cells which contain metachromatic granules.^{4,6,7} The amnion contains some mast cells, while the placental decidua is apparently devoid of them.^{3,6,10} Mast cells seem to be present in great number in Wharton's jelly of the umbilical cord, and some anticoagulant properties have been attributed to extracts of both cord and placenta, this quality being ascribed to the presence of some chondroitinsulfate compound.^{5,6,9} In all probability the mast cells form this product. It has been suggested that the metachromatic granules possess some anticoagulant action on the basis of staining reactions, indicating that they contain heparin or some heparin-like substance.⁴ Mast cells may also contain histamine.⁸ Previous histochemical studies of the placenta have indicated a paucity or complete lack of mast cells residing in the area between amnion and chorion.^{4,6,7}

The area between amnion and chorion was investigated, microscopically, by the following procedure: Within two hours post partum, the fetal side of the placenta was washed gently with tap water and then blotted dry. A small piece of amnion (usually about 2×4 cm.) was then stripped from the area overlying the placenta. The underlying chorionic plate was removed. Both tissues were placed in a one-half saturated solution of methylene blue (polychromed) in 50% ethyl alcohol, stained for one minute, and then washed in water and mounted

in Permount after slight air drying. In several instances the amnions were stained, using a 0.25% solution of toluidine blue in 70% alcohol at pH 2. The tissues were mounted in such a manner that the mucoid-material-containing area between amnion and chorion was "up" for both of the specimens. A total of 50 "full-term" placentas were examined, in addition to a number of younger ones of varying ages.

Upon examination of the amnions, it could be seen that in the layer farthest from the epithelial surface, and apparently in one focal plane, there were many cells containing metachromatic granules, demonstrable with both staining techniques. The nuclei were pale, oval, and clear to slightly granular, with no nucleoli noted (Figs. 1 and 2).

The metachromatic granules varied markedly in size and density from one specimen to another, with minor variations noted from one area to another of the same placenta. This variability possibly represents a difference in hydration.⁵ In all instances, aside from those noted below, these cells were observed on the chorionic side in number equal to or greater than that seen on the amniotic side. The main difference between the two was that the cells containing the metachromatic granules were not located in a single focal plane on the chorionic side but, rather, were distributed throughout the mucoid substance in variable concentrations. In five instances, extremely few or no cells were seen on the portion of the amnion examined, though its corresponding chorion contained normal numbers. In another 5 of the 50 full-term placentas, the content of mast cells in both amnion and chorion was markedly diminished. In no instance could we find any common variation from the other "normal" placentas to account for this difference, neither with the gross and

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From the Department of Obstetrics and the Department of Pediatrics, University of Cincinnati College of Medicine; the Laboratory for the Study of Infant and Maternal Health, and the Children's Hospital Research Foundation.

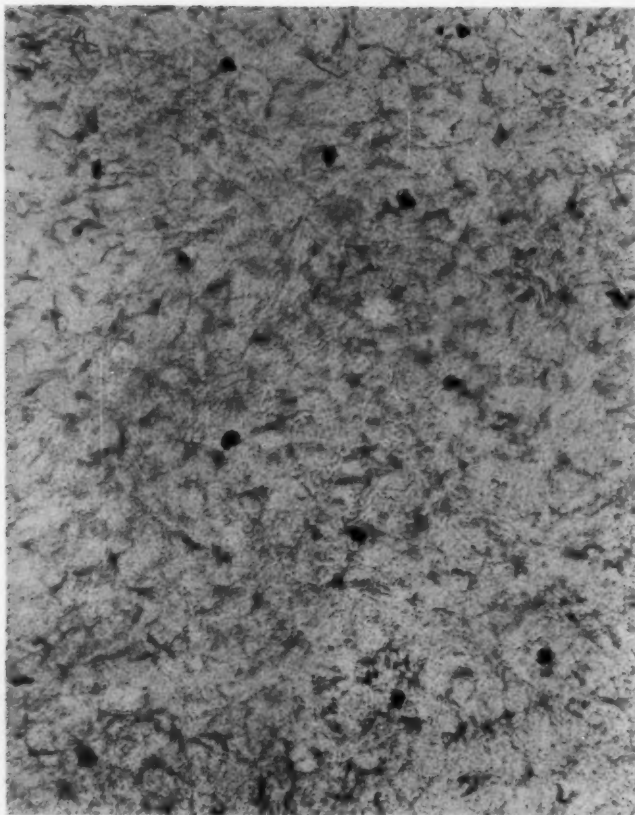


Fig. 1.—Methylene blue-stained amnion showing mast cells lying in one focal plane. $\times 150$.

routine microscopic examination of the placentas nor with the clinical and laboratory evaluation of the pregnancy, the mother, and the child.

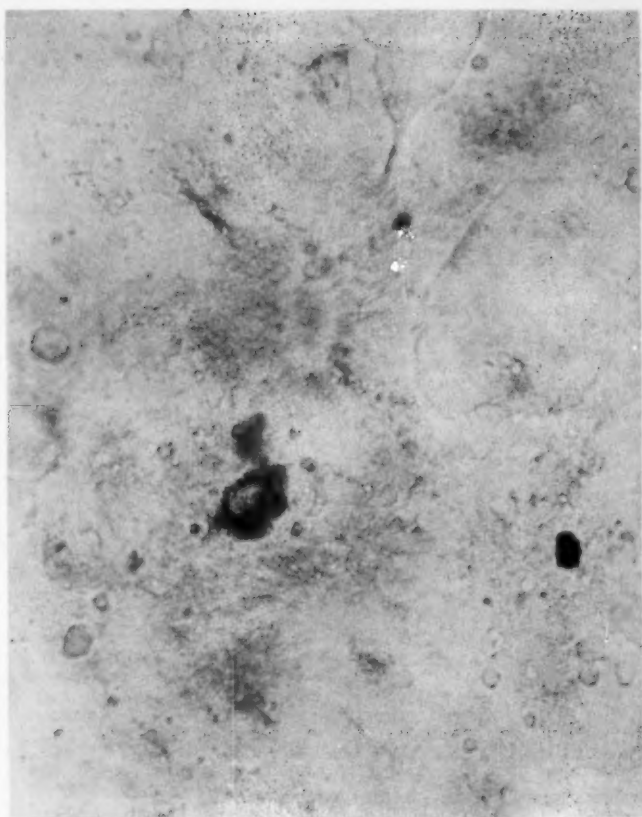
The placenta of a patient who had been receiving desiccated thyroid for a severe hypothyroid condition was noteworthy. Very few mast cells were found. This might have been suspected if Asboe-Hansen's conclusions are correct, i.e., that there is an increased number of mast cells in myxedematous connective tissue, which diminishes as the TSH level is brought down by the administration of thyroid hormone.^{1,2} The infant of this case was circumcised on the seventh day post partum. The prepuce showed an apparently normal concentration of mast cells, though he was taking $\frac{1}{4}$ grain (15 mg.) of desiccated thyroid daily and had no clinical or laboratory (insensible

weight loss, PBI) evidence of myxedema at the time.

Placentas of premature and stillborn infants were also examined. While mast cells are described as being present in villi of placentas as young as 3.5 weeks, we were unable to demonstrate them in the amnion or chorion prior to the 20th week of gestation. After this age, characteristic mast cells could be found if a large enough area was explored (on occasion up to half the placental surface).

The mast cells found in placental villi possibly actually do act to inhibit clotting of maternal blood by release of some anticoagulant substance into the area.⁴ It is difficult to say whether or not the mast cells described above have the same proximate action, such as, for example, to inhibit formation of subchorionic thrombi, or

Fig. 2.—Higher power photomicrograph of Figure 1 with a single mast cell in the field. $\times 650$.



whether this is to be considered as merely another connective-tissue area of the body in which the presence of mast cells in good number might have been anticipated.

Children's Hospital Research Foundation, Elland and Bethesda Aves. (Dr. Sutherland).

REFERENCES

1. Asboe-Hansen, G.: The Origin of Synovial Mucin: Ehrlich's Mast Cell—a Secretory Element of the Connective Tissue, *Ann. Rheumat. Dis.* 9:149-157, 1950.
2. Asboe-Hansen, G., in *Transactions of the 5th Conference on Connective Tissues*, New York, The Josiah Macy Jr. Foundation, 1954.
3. Ferroni, E.: Sulla presenza e sulla distribuzione delle cosiddette "mastzellen" nella membrana amnios, *Arch. ital. ginecol.* 1:447, 1898, as noted by N. A. Michels in *Handbook of Hematology*, edited by H. Downey, 1938, Vol. 1, pp. 232-272.
4. Latta, J. S., and Beber, C. R.: Cells with Metachromatic Cytoplasmic Granules in the Stroma of Human Chorionic Villi, *Science* 117:498-499, 1953.
5. Moore, R. D.: Mast Cells of the Human Umbilical Cord, *Am. J. Path.* 32:1179-1183, 1956.
6. Pagani, C.: I mastociti della placenta e del cordone ombelicale umani in condizioni fisiologiche: Studio istochimico e istomorfologico, *Ann. ostet. gin.* 74:429-442, 1952.
7. Pescetto, G.: Sulla presenza di elementi granulosi basofili metacromatici nella placenta fatale umana, *Biol. lat.* 2:744-757, 1950.
8. Riley, J. F.: Pharmacology and Functions of the Mast Cells, *Pharmacol. Rev.* 7:267-277, 1955.
9. Sundberg, R. D.; Schaar, F. E.; Powell, M. J. S., and Denboer, D.: Tissue Mast Cells in Human Umbilical Cord, and the Anticoagulant Activity of Dried Extracts of Cords and Placentae, *Anat. Rec.* 118:35-56, 1954.
10. Wislocki, G. B., and Dempsey, E. W.: The Chemical Histology of the Human Placenta and Decidua with Reference to Mucopolysaccharides, Glycogen, Lipids and Acid Phosphatase, *Am. J. Anat.* 83:1-41, 1948.

Beber et al.

The Erythrocyte Acetylcholinesterase Enzyme in Paroxysmal Nocturnal Hemoglobinuria

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With the Technical Assistance of Margaret C. Carl

In an earlier communication we reported that erythrocyte acetylcholinesterase (AChE)* activity was subnormal in eight patients with paroxysmal nocturnal hemoglobinuria (PNH)† studied.¹ To our knowledge, this has been the only enzyme defect observed in the PNH erythrocyte. De Sandre, Ghiotto, and Mastella reported a similar finding in one PNH patient.² With biochemical methods Beck and Valentine noted that the alkaline phosphatase activity in PNH neutrophilic leukocytes was reduced,³ and this was confirmed in four PNH patients, using a histochemical method.⁴ Although there is evidence to suggest that glycolysis is normal in PNH erythrocytes,^{1,5} a decreased uptake of radioactive phosphorus and an increased turnover of adenosinetriphosphate (ATP) has been observed.^{6,7} On the other hand, potassium transport has been found to be normal in PNH cells.⁸ Several workers have reported erythrocyte lipid abnormalities,⁹⁻¹¹ and electron microscopy has revealed a patchy and pitted erythrocyte surface.¹²⁻¹⁴ The reason for the

markedly reduced AChE activity remains obscure. The present investigation was designed to extend our earlier studies and to explore the nature of this subnormal enzyme activity in PNH erythrocytes.

Material and Methods

Patient Material.—Cases 1 to 6 were studied at Vanderbilt University Hospital.⁴ The red cells of Case 7 were provided by Dr. C. Lockard Conley, The Johns Hopkins School of Medicine; those of Cases 8 and 9, by Dr. Carl F. Hinz Jr., Western Reserve University School of Medicine; those of Case 10, by Dr. William H. Crosby, Walter Reed Army Medical Center, and those of Case 11, by Drs. Stanley B. Troup and Lawrence E. Young, University of Rochester School of Medicine and Dentistry. All but Case 10 had clinically active PNH. Although in the past this patient had had clinically active disease, at the time of study blood counts and red cell survival were normal, and the acid hemolysis test was only weakly positive.¹⁵ Data regarding this patient have been previously published.¹⁶

Unless otherwise specified, the erythrocyte enzyme studies reported refer to specimens obtained at least two months following the last transfusions. In most instances the interval was much longer. Cases 2 and 10 have never had transfusions.

Preparation of Packed Erythrocyte Specimens.—Blood was collected without the use of anticoagulant from an antecubital vein into silicone-treated tubes and centrifuged at high speed ($10,000\times g$) in a refrigerated centrifuge for 10 minutes.¹ The plasma was carefully removed, and top, middle, and bottom layers were isolated from the packed red-cell column. A "mixed" red-cell sample, representative of cells at all levels of the column, was prepared by taking duplicate packed red cell columns, mixing them thoroughly, and obtaining an aliquot from this mixture. The cells were then washed three times with large volumes of 0.85% sodium chloride solution and finally packed again by centrifuging at 3,000 rpm for

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* The abbreviation AChE will be used throughout the paper.

† The abbreviation PNH will be used throughout the paper.

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eight minutes. The enzyme activity of 0.2 ml. portions of washed packed cells was determined.

Reticulocyte Counts.—Reticulocyte counts were made on dry films after vital staining with brilliant cresyl blue. All erythrocytes containing recognizable reticulum were counted, and the percentage of reticulocytes in 1,000 erythrocytes was determined. It was necessary to dilute the various specimens of packed cells in the original plasma for reticulocyte counts, since saline suspension proved unsatisfactory.

"In Vitro" Cell Mixtures.—Fresh washed and packed normal and PNH cells were mixed with each other in varying proportions. PNH red cells were also mixed in varying proportions with cells isolated from a patient with pernicious anemia recently started on cyanocobalamin U.S.P. (vitamin B₁₂) therapy.

Transfusion Studies.—Blood was freshly collected from each of two normal donors into Fenwall plastic bags containing acid citrate-dextrose solution. The donor blood was compatible with the recipient PNH patient's blood by saline, albumin, and indirect Coombs cross-matching techniques. The donor red cells were washed three times with 0.85% sodium chloride solution, and the cells were packed by centrifugation at 3,000 rpm for 30 minutes. The cells were then tagged with Cr⁵¹ (as sodium chromate) by injecting 65 μ c. of the latter into each bag, followed by thorough mixing and incubation overnight at 5 C. One hundred milligrams of ascorbic acid was then added to each bag, and the contents of the two were pooled into one bag.

Just prior to infusion of the normal packed cells into the PNH patient, samples were removed both from the normal cells and from the recipient PNH patient for AChE determinations and to serve as standards for radioactivity counting. Radioactivity was determined by scintillation-counting techniques.

The normal tagged cells were infused into the PNH patient over a two-hour period. Thirty minutes later blood samples were obtained from the patient for AChE and radioactivity determinations. These tests were carried out on the various layers of a packed-cell column from each sample. All final samples were weighed so that radioactivity could be expressed in counts per second per gram of cells. From these data the proportion of infused normal cells in the various erythrocyte layers of post-transfusion recipient blood was calculated.

Determination of Acetylcholinesterase Activity.—Acetylcholinesterase activity was determined manometrically by employing the Warburg technique. Measurements were carried out at 37.5 C in Ringer-Krebs bicarbonate medium. The final volume was 2.0 ml. Activity-pS curves were determined for

normal cells, and the optimal substrate concentration was found to be approximately 1.5×10^{-3} M. This substrate concentration gave slightly higher values for enzyme activity of normal human erythrocytes than did concentrations previously used.¹ The substrate employed was acetylcholine bromide which had been recrystallized several times from absolute alcohol. The vessels were gassed with 95% N₂ and 5% CO₂ for 20 minutes. The substrate and cells were mixed, and exactly three minutes was allowed for reequilibration. Successive manometric readings were made at intervals of 5 minutes for a total of 30 minutes. The first 10-minute interval was considered to be the most accurate measurement. This value was multiplied by 6 to give the rate per hour.

Although a volume measurement of packed cells was used for the determinations, final expression of enzyme activity was given in terms of dry weight. This was preferred, since the volume of the reticulocyte is known to be appreciably greater than the volume of the mature red cell.¹⁷ (Allison and Burn expressed their results in terms of a unit quantity of hemoglobin, since this substance "is probably metabolically inert in the mature erythrocyte, and the quantity of hemoglobin in any one cell should not vary appreciably with age."¹⁸ On the other hand, although the mean corpuscular concentration of reticulocytes is lower than that of mature erythrocytes in experimental hemolytic anemia in rabbits, the absolute quantity of hemoglobin in the reticulocyte was reported as increased.¹⁷) Allison and Burn also emphasized that reference of activity to a unit number of red cells is not reliable because of the large random error in red cell counts.¹⁸ In our studies dry weights determined on a unit volume of top, middle, and bottom layers were actually very nearly the same.

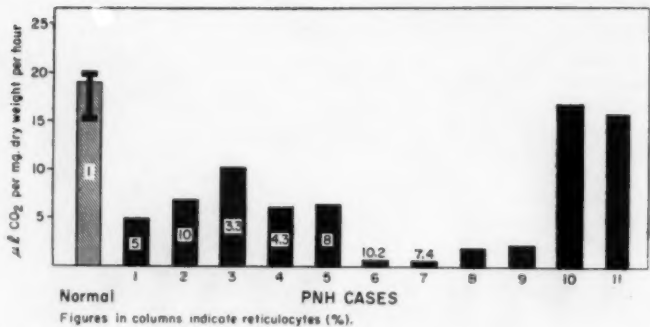
Dry Weights: Two-tenths milliliter of the packed red cells was pipetted carefully into small tin-foil cups. The cups were weighed empty and reweighed after the red cell content reached a constant weight in a drying oven set at 110 C.

Results and Comment

Erythrocyte Acetylcholinesterase Activity in Paroxysmal Nocturnal Hemoglobinuria (PNH)

Representative data regarding erythrocyte acetylcholinesterase (AChE) activity in 11 PNH patients and in normal subjects are given in Figure 1. The values are for "mixed layers," representative of the entire population of red cells, and the figures in the columns indicate the percentage of

Fig. 1.—Acetylcholinesterase activity of normal and PNH erythrocytes. The I-beam superimposed on the column for normal erythrocytes indicates the range of values.



reticulocytes. The data for normals were obtained from one or more determinations one each of 20 normal subjects. The enzyme activity in PNH Cases 1-9 was markedly reduced, and all of these patients had clinically active disease.⁴ The normal AChE activity in Case 10 may possibly be associated with current inactivity of the hemolytic disorder, as substantiated by normal blood counts and red cell survival time.¹⁵ In this patient the acid hemolysin test is at present only weakly positive. Such, however, would not explain the normal enzyme activity in Case 11, who has active disease.¹⁹ The acid hemolysin test has been clearly and repeatedly positive on numerous occasions in this patient.¹⁰ Furthermore, she has never received blood transfusions.

Dr. Sabine, of the University of California Medical Center, has kindly given us permission to report the erythrocyte acetylcholinesterase activity in another PNH case. The red cell cholinesterase activity

of this PNH case was 0.45 when referred to the mean normal value of 1.00, expressed per unit volume of red cells.

Erythrocyte Age and Acetylcholinesterase Activity

The higher proportion of reticulocytes in the top layer as against the bottom layer of packed red cells is an expression of the fact that the top layer contains the younger red cells. Normally in both humans and rats the top layer has been noted to contain greater AChE activity than the bottom layer.^{20,21} Allison and Burn concluded that in normal blood the AChE activity of reticulocytes is about three times as great as that of mature erythrocytes.¹⁸

On the other hand, the immature cells in PNH generally showed just as much impairment of enzyme activity as the more mature cells. Figure 2 shows representative data of AChE determinations in the top

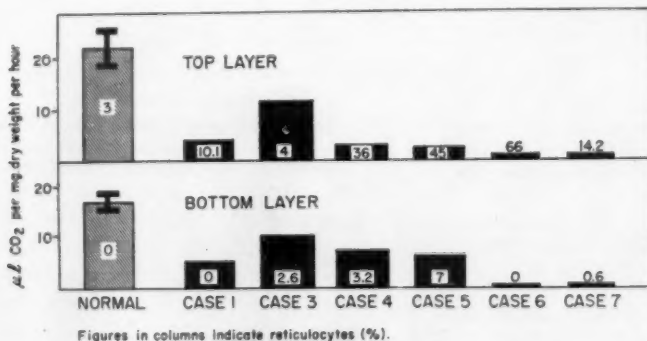


Fig. 2.—Acetylcholinesterase activity in top and bottom layers of normal and PNH erythrocytes. The I-beams superimposed on the columns for normal erythrocytes indicates the range of values.

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and bottom layers of six PNH cases. In most instances the enzyme activity of the immature (top layer) cells was actually less than that of the mature (bottom layer) cells. The studies in Case 6 are of particular interest, since, despite the fact that the top layer consisted of 66% reticulocytes, there was virtually no AChE activity.

Further evidence that the immature erythrocytes possessed the enzyme defect was provided by preliminary marrow studies. Enzyme activity of the cellular portion of bone-marrow aspirates in Cases 3 and 4 was even lower than that of peripheral blood red cells simultaneously determined. In view of the cellular diversity of bone-marrow aspirates, definitive studies will require histochemical determination of AChE in marrow smears or sections.

Fluctuation of AChE Activity in PNH

Earlier studies in our laboratory suggested that erythrocyte AChE activity in normal subjects and PNH patients fluctuated from time to time.¹ The range of variation in normal subjects is shown in Figure 1. At no time did the values for the Vanderbilt PNH patients (Cases 1-6) attain normal levels of activity. Since the initial observations, we have determined the enzyme activity in Cases 4-6 on many occasions. Table 1 provides representative data of erythrocyte enzyme values in Case 5 obtained daily for one week and in Case 6

values obtained during a five-month period. No transfusions were given during these periods. There was very little fluctuation in enzyme activity in either patient. The degree of fluctuation noted might be explained by the technical variability of the enzyme determinations alone. There was also no significant alteration during hemoglobinuric episodes, and in other studies (not shown) no variations in daytime and nighttime AChE activity.

Possibility of an Inhibitor in PNH Erythrocytes

Prolonged incubation of normal red cells with PNH serum and PNH cells with normal serum did not affect the respective cellular enzyme activities. This provides evidence against the presence of a circulating inhibitor of erythrocyte AChE in PNH.

Heating of Erythrocytes.—PNH cells were heated at 56 C for one hour. At this temperature the AChE activity was still further reduced, but addition of these cells to normal erythrocytes did not affect the activity-pS curves of the latter. Thus, no heat-stable inhibitor could be demonstrated in PNH cells. In similar experiments no heat-stable activator for the depressed AChE activity in PNH cells could be demonstrated in the red cells of a patient with pernicious anemia whose erythrocyte AChE activity was elevated during the period of reticulocytosis following cyanocobalamin therapy.

In studies exploring the possible existence of a heat-labile inhibitor of AChE, PNH cells were mixed in varying proportions with normal cells and with pernicious anemia cells with elevated activity following cyanocobalamin therapy. These mixtures were incubated for 15 minutes at 37 C prior to enzyme determinations. The activity of such mixtures was essentially the algebraic sum of the activities of the separate specimens. Sabine pointed out that such experiments are conclusive only at optimal substrate concentrations, and that they neither establish nor disprove the presence of a competitive inhibitor.²¹ Sabine also observed

TABLE 1.—Variation in Erythrocyte AChE at Different Times

Date	Normal	PNH Case 5	Date	Normal	PNH Case 6
1958					
Oct. 8	18.6*	5.3	July 25	20.0	1.2
9	17.8	6.1†	Aug. 1	16.9	3.2
10	19.0	5.5	9	17.0	2.5
11	18.0	7.0†	14	19.0	1.1
12	19.1	5.8	22	20.0	1.5
13	16.9	6.9	23	18.0	1.1
14	17.8	6.1†	29	16.9	0.9
			Oct. 29	18.8	1.0
			Nov. 3	19.0	2.0
			Dec. 9	18.1	0.5

* Microliter (μl.) CO₂ per milligram dry weight per hour.

† Hemoglobinuria present.

similar findings working with red cells isolated from a patient with pernicious anemia during both relapse and treatment.²¹ She concluded that the content of whatever activators or inhibitors may be present in pernicious anemia cells are not much different than those present in normal cells. Similar conclusions regarding PNH cells were reached from the results of our studies.

Effect of Pyridine-2-Aldoxime (2-PAM).

AChE can be inhibited by low concentrations of certain organophosphorus compounds, such as isofluorophate, tetraethylpyrophosphate (TEPP), and isopropoxymethylphosphonofluoride (Sarin). Inhibition is accomplished by phosphorylation of the enzyme. The inhibited enzyme can be dephosphorylated and reactivated by such nucleophilic agents as pyridine, imidazole, and hydroxylamine.²² Grob and Johns found that the oxime pyridine-2-aldoxime methiodide restored to a moderate degree the activity of human erythrocyte AChE which had been inhibited by organophosphorus anticholinesterase compounds.²³ Re-activation of the inhibited enzyme proceeded rather slowly. PNH erythrocytes from Cases 4, 5, and 6 were incubated at 37 C with concentrations of 2-PAM ranging from 5×10^{-2} to 5×10^{-6} M for varying periods of time (1 to 48 hours). No change in the subnormal level of PNH erythrocyte AChE activity was noted.

Freezing and Thawing.—Freezing and thawing is a technique frequently employed to aid in the purification of enzymes. This procedure was used in an attempt to destroy or release any tightly bound inhibitor of AChE. Rapid freezing and thawing of PNH red cells for as many as 27 times did not alter the subnormal enzyme activity.

"In Vivo" Mixture of Normal and PNH Erythrocytes (Transfusion Experiments)

The well-known finding that a transfusion of washed red cells frequently abolishes a hemoglobinuric crisis was verified in our experience.⁴ For several weeks following such a transfusion the bouts of hemoglobinuria may cease or decrease in frequency. Others have suggested that transfusions not only halted the hemoglobinuria but decreased the absolute number of cells "susceptible" to in vitro hemolysis in acidified serum.²⁴ Furthermore, our earlier studies suggested that the rise in AChE in cells obtained from PNH patients following transfusions might be greater than that expected from simple mixing of the two cell populations.¹ Thus the possibility existed that normal cells might in some manner increase the enzyme activity in the PNH cells. For this reason transfusion experiments were carried out with normal washed cells tagged with Cr⁵¹.

Table 2 shows the results of one experiment. (Data of the second experiment are

TABLE 2.—*Influence of Transfusion of Normal Red Cells on PNH Erythrocyte AChE*

Packed Cell Sample	Donor Cells	Cells from PNH Case 5			Theoretical Post-Transfusion Erythrocyte AChE in PNH Patient *
		Before Transfusion	After Transfusion of Two Units of Normal Cells	Infused Normal Cells in Layers	
	AChE †	AChE	AChE	%	AChE
Top layer	22.1	1.0	3.7	11.2	3.4
Middle layer	18.0	4.6	7.7	--	--
Bottom layer	17.3	5.6	10.6	36.1	9.8
Volume of packed red cells in peripheral blood		20.0 %	28.2 %		

* Data calculated from AChE values for donor cells, pretransfusion PNH cells, and percentage of normal infused cells (Cr⁵¹ tagged) in respective layers. Compare with manometrically determined post-transfusion AChE values in PNH patient.

† Microliter CO₂ per milligram dry weight per hour.

not given, since the results were virtually the same.) After transfusion of 2 units of washed red cells, the tagged normal cells were not found equally distributed in the top and bottom layers. The top layer consisted of 11.2% normal transfused cells and 88.8% patient's own red cells. The distribution for the bottom layer was 36.1% normal cells and 63.9% patient's own cells. This unequal distribution of infused normal cells can be explained by the higher proportion of older red cells in normal blood than in PNH blood, in which the red cell life span is ordinarily greatly shortened.

AChE activity was determined in top, middle, and bottom layers of samples from the tagged donor cells and from the PNH patient before and after transfusion. The far right-hand column in Table 2 shows the theoretic calculated value of erythrocyte AChE in the transfused PNH patient based on the assumption that this value is the result of simple mixing. These values were in good agreement with the manometrically determined AChE activity in the post-transfusion cells (3.4 compared with 3.7 for the top layer and 9.8 compared with 10.6 for the bottom layer). Thus the increase in AChE activity after transfusion could be explained by simple algebraic summation of the activities of PNH and normal red cells determined separately. These findings are similar to those obtained with *in vitro* cell mixing experiments. From these studies it appears unlikely that PNH cells differ significantly from normal cells in whatever content of activators or inhibitors of AChE they may contain.

The demonstrated preferential localization of transfused normal cells in the bottom layer of samples removed from PNH patients has further implications. Previously reported erythrocyte AChE values for Case 6 were higher than those recorded in the present communication, particularly in bottom-layer erythrocytes.¹ The earlier samples were virtually all obtained during a period of two to four months following transfusions. Since this period falls within the life span of normal cells, an appreciable number

of normal transfused cells may have contaminated the bottom-layer cells. Erythrocyte specimens obtained when Case 6 was free of all transfused cells (i.e., more than four months after the last transfusion) have usually revealed virtually no AChE activity. For practical purposes it is impossible to delay transfusions in most PNH patients for such long periods. Therefore, the activity of the top layer of PNH cells in the transfused patient should ordinarily be considered a more accurate reflection of the true enzyme value for the PNH cell than the bottom layer or a mixed-cell sample. In any hemolytic disorder severe enough to require transfusions, the best reflection of the status of the patient's own cells should be obtained from the top layer of cells centrifuged at high speed. Such a principle may apply to serological, as well as to biochemical, tests.

Genetic Studies

In enzyme deficiency states there always lurks the suspicion of heritable transmission. However, PNH has not been detected in family members of patients.²⁵ Dameshek reported PNH in one of identical twins, the other of whom remained well.²⁶ Yet if gene penetrance is as low as 20%-30% or simple Mendelian recessive inheritance is present, it may be extremely difficult to demonstrate heritable transmission, particularly in a disorder as rare as PNH.

Erythrocyte AChE activity, clinical histories, and blood counts were normal in three family members of Case 4 (two siblings and one child) and in nine of Case 5 (both parents, four siblings, and three children). In instances of incomplete gene dominance, family members may have "low-normal" biochemical values for the defect. However, our preliminary studies suggest that the "scatter" of AChE values in family members was in the same range as the "scatter" for normal control subjects. Studies on family members of other PNH patients are in progress.

Available information suggests that the erythrocyte AChE enzyme may be similar to the AChE enzyme of nerve cells. However, there has been no clinical evidence to suggest deficient AChE activity of nerve cells in PNH, and direct biochemical studies are not feasible.

The neurological complications occasionally seen in PNH have usually been ascribed to vascular accidents.²⁵ Nevertheless, such evidence does not exclude genetic transmission, since theoretically alternate metabolic pathways could compensate for and mask the effect of a genetic defect in the nerve cells. Furthermore, *somatic* mutation restricted to the marrow erythroblasts remains possible.

Nature of the Acetylcholinesterase Defect

The subnormal erythrocyte AChE activity could be the result of one factor or of a combination of several factors. It may be due to (1) reduced protein synthesis, (2) destruction, (3) inhibitor produced at the site of red-cell genesis, or (4) the manufacture by the bone marrow of a qualitatively abnormal enzyme.

Although our studies do not necessarily prove or disprove the presence of a competitive inhibitor of AChE in PNH erythrocytes, they suggest that some biochemical abnormality present in the bone marrow results in the formation of defective cells. The presence of a noncompetitive or tightly bound inhibitor produced in the bone marrow also has to be considered, even though our studies provided no support for this concept. Enzyme purification studies should supply valuable information regarding the presence or absence of such an inhibitor. Studies of this type are now in progress.

The possibility also exists that the structure of the enzyme is abnormal. The active site of the AChE enzyme which is responsible for hydrolyzing acetylcholine is thought to be divided into two subsites (anionic and cationic).²⁷ It is possible that in PNH cells one or both sites may be physically

masked or distorted as a consequence of abnormal protein structure.

The most attractive hypothesis consonant with available knowledge is that decreased enzyme or protein synthesis at the site of red cell production best explains the low AChE activity in PNH cells. This concept could be related to the electron microscopy observation that the PNH red cell membrane is patchy and pitted.¹²⁻¹⁴ Since AChE is situated in the red cell membrane, the patches and pits may represent the absence of this enzyme. Cheli and Dianzani noted both a reduction in erythrocyte AChE and "irregular roughness and porosity" of the red cell membrane in experimental immune hemolytic anemia in guinea pigs.^{28,29}

Abnormal protein synthesis could be due to a number of factors. For example, certain nucleic acids are thought to be intimately involved in protein synthesis. Crosby has suggested that PNH may be due to a permanent alteration of those parts of the reticuloendothelial system that produce the stromal proteins of blood cells.²⁵ Certain experiments with bacteriophage have suggested that infection of the host cell impairs its function in the synthesis of proteins (enzymes), perhaps by virtue of altering nucleic acid metabolism.³⁰ Viral etiology in PNH has not been eliminated.²⁵ It remains possible that a virus could alter nucleic acid in PNH and thereby lead to abnormal protein synthesis.

Significance of the Enzyme Defect

It is obviously difficult to assess the significance of a defect in erythrocyte AChE in relation to the hemolysis of PNH cells when the physiological role of the enzyme itself is not fully understood. The equilibrium between acetylcholine and its hydrolytic products

$\text{Acetylcholine} + \text{H}_2\text{O} \rightleftharpoons \text{Acetic Acid} + \text{Choline}$
is dependent of pH.³¹ Regarding nerve tissue, it has been suggested that the acetylcholine-cholinesterase system acts as a buffer in the nerve membrane, catalyzing the formation of acetylcholine as H^+ ions accum-

ulate during the conductive process.²⁷ It remains possible that AChE plays a similar role in the erythrocyte, but since there is no conclusive evidence that red cells can synthesize acetylcholine *in vivo*, the function of erythrocyte AChE remains obscure.³¹ It is conceivable that the erythrocyte enzyme may prevent the local accumulation of H^+ -ions (such as would result from glycolysis or other metabolic processes), which, in turn, would damage the cell, possibly by altering the membrane permeability.³¹ An earlier hypothesis that erythrocyte AChE governs potassium transport³²⁻³⁴ could not be substantiated in certain studies with enzyme-deficient PNH cells, since potassium transport was normal in such cells.⁸

Erythrocyte AChE activity can be inhibited by physostigmine. Working with such drug-inhibited cells suspended in isotonic solutions of choline esters, Holland and Graham concluded that the ability of mammalian erythrocytes to withstand the damaging effects of an increased H^+ -ion concentration was dependent on the AChE content of the red cell membrane.³⁵ It was thought that the enzyme system acted as a buffer, in the manner suggested by Bergmann and Shimoni.²⁷ This hypothesis would provide a neat explanation for the susceptibility of the AChE-deficient PNH erythrocyte to hemolysis when the pH of the serum is lowered. However, employing the experimental design of Holland and Graham, we were unable to obtain evidence for this hypothesis when PNH cells were suspended in isotonic solutions of choline esters.³⁶ The high concentrations of acetylcholine used in such studies are unphysiological. Furthermore, definitive studies must include experiments with PNH cells suspended in *serum* and attempts to assess the buffer capacity of PNH cells and stroma.

The subnormal erythrocyte AChE in PNH may be merely a reflection of decreased stromal protein synthesis. Subnormal stromal protein concentration itself may result in reduced buffer capacity of the erythrocyte membrane and hence impaired

resistance to the damaging effects of increased H^+ -ion concentration.

Torp demonstrated in rats that the splenic blood pH was lower than that of vena cava blood and suggested that, therefore, the spleen may provide ideal conditions for physiological hemolysis.³⁷ Thus this low pH would provide the maximum opportunity for hemolysis of PNH erythrocytes. However, splenectomy has had little or no beneficial effect on hemolysis in PNH.²⁵ Furthermore, Torp also provided evidence that the low pH in rat splenic blood was due to the enzymatic hydrolysis of splenic acetylcholine by the AChE of the erythrocytes sequestered in this organ. Even though the AChE-deficient PNH cells would theoretically produce fewer H^+ -ions, such cells, on the basis of the enzyme buffer system concept,³⁵ might be more susceptible to H^+ -ions produced by other metabolic processes.

From a clinical standpoint the absence of alteration in enzyme activity during hemolytic crisis and of any significant diurnal variation suggests that the AChE deficiency may not be fundamentally concerned with the hemolysis of PNH cells. However, the proportion of cells "susceptible" to hemolysis at any given time may be relatively small (3%-33%).³⁸ Any further reduction in enzyme activity in the very small proportion of "susceptible" cells may not be detectable when the entire population of red cells is analyzed for AChE activity by the presently available methods.

Despite normal erythrocyte AChE activity Case 11 had "persistent hemoglobinemia and frequent bouts of hemoglobinuria, especially after being in bed for 6 to 8 hours." Furthermore, Case 6, with the lowest erythrocyte AChE activity, has had a relatively benign course with only four episodes of hemoglobinuria in nine years.⁴ Her transfusion requirements have been minimal. Following the correction of iron deficiency, the volume of packed red cells has stabilized at 30%-34%.

There is also no clinical evidence that inhibition of erythrocyte AChE activity

results in a hemolytic disorder. Such has apparently not been noted in experimental and accidental poisoning with insecticides, such as Parathion.^{39,40} Although jaundice has been noted, there has been no evidence to suggest any increased hemolysis.³⁹

AChE deficiency is probably but one facet in a constellation of interrelated biochemical abnormalities in the PNH erythrocyte. Therefore, judgment should be reserved regarding the role of deficient AChE activity in the hemolysis of PNH cells until the relationship of the enzyme to erythrocyte membrane pH and to the serum hemolytic system can be clarified. Such studies should also shed light on the physiological role of the enzyme relative to the erythrocyte.

Summary

Subnormal erythrocyte acetylcholinesterase activity is reported in 10 of 12 patients with paroxysmal nocturnal hemoglobinuria. One of the patients with normal enzyme activity is in spontaneous remission, but the other has clinically active disease.

The acetylcholinesterase defect was present in immature, as well as mature, erythrocytes.

There was no significant diurnal variation in erythrocyte acetylcholinesterase activity, nor was there any change during hemoglobinuric crisis.

Both in vitro and in vivo studies provided no evidence of any inhibitor of acetylcholinesterase in the PNH erythrocyte.

The nature and significance of the deficient acetylcholinesterase activity in PNH erythrocytes are discussed.

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Department of Medicine, Vanderbilt University School of Medicine.

Addendum

Recently De Sandre and Ghiotto reported reduced erythrocyte acetylcholinesterase activity in six of seven patients with PNH.⁴¹ Barry reinvestigated four of the six patients of Harris and his associates¹⁰ and reported normal phospholipids in PNH red cells.⁴²

REFERENCES

1. Auditore, J. V., and Hartmann, R. C.: Paroxysmal Nocturnal Hemoglobinuria: II. Erythrocyte Acetylcholinesterase Defect, *Am. J. Med.* 27:401, 1959.
2. De Sandre, G.; Ghiotto, G., and Mastella, G.: L'acetilcolinesterase eritrocitaria: II. Rapporti con le malattie emolitiche, *Acta med. patav.* 16:310, 1956.
3. Beck, W. S., and Valentine, W. N.: Biochemical Studies on Leucocytes: II. Phosphatase Activity in Chronic Lymphatic Leukemia, Acute Leukemia, and Miscellaneous Hematologic Conditions, *J. Lab. & Clin. Med.* 38:245, 1951.
4. Hartmann, R. C., and Auditore, J. V.: Paroxysmal Nocturnal Hemoglobinuria: I. Clinical Studies, *Am. J. Med.* 27:389, 1959.
5. Pranker, T. A. J.: The Metabolism of the Human Erythrocyte: A Review, *Brit. J. Haemat.* 1:131, 1955.
6. Hellem, A. J., and Skaug, O. E.: Paroxysmal Nocturnal Hemoglobinuria: II. Permeability and Phosphate Turnover in the Red Blood Cells, *Scandinav. J. Clin. & Lab. Invest.* 7:121, 1955.
7. Altman, K. I.; Tabechian, H., and Young, L. E.: Some Aspects of the Metabolism of Red Blood Cells from Patients with Hemolytic Anemias, *Ann. New York Acad. Sc.* 75:142, 1958.
8. Auditore, J. V.; Hartmann, R. C., and Cole, E. F.: Potassium Transport in the Acetylcholinesterase-Deficient Erythrocytes of Paroxysmal Nocturnal Hemoglobinuria, *J. Clin. Invest.* 38:702, 1959.
9. Rodbard, J. A.: Chronic Hemolytic Anemia with Nocturnal Hemoglobinuria (van Marchiafava-Micheli), Thesis, University of Amsterdam, Amsterdam, 1950.
10. Harris, I. M.; Pranker, T. A. S., and Westerman, M. P.: Abnormality of Phospholipids in Red Cells of Patients with Paroxysmal Nocturnal Hemoglobinuria, *Brit. M.J.* 2:1276, 1957.
11. Munn, J. I., and Crosby, W. H.: Paroxysmal Nocturnal Hemoglobinuria: Evidence of Defect

ACETYLCHOLINESTERASE IN PNH ERYTHROCYTES

of Red Cell Stroma Manifested by Abnormalities of Lipids, *Proc. Soc. Exper. Biol. & Med.* 96: 480, 1957.

12. Matthes, M.; Schuboth, H., and Lindemann, B.: Klinische und experimentelle Studien zur chronischen hämolytischen Anämie mit nächtlicher Hämoglobinurie (Typ Marchiafava-Micheli), *Acta haemat.* 5:193, 1951.

13. Braunsteiner, H.; Gisinger, E., and Pakesch, F.: Confirmation of Structural Abnormality in Stroma of Erythrocytes from Paroxysmal Nocturnal Hemoglobinuria After Hemolysis in Distilled Water, *Blood* 11:753, 1956.

14. Cecchi, E., and Conestabile, E.: Paroxysmal Nocturnal Hemoglobinuria: Electron-Microscopic Study of Red Blood Cells, *Lancet* 2:466, 1957.

15. Crosby, W. H.: Personal communication.

16. Blount, R. E.: Chronic Hemolytic Anemia with Paroxysmal Nocturnal Hemoglobinuria: Case Report, *Am. Pract. & Digest Treat.* 4:768, 1953.

17. Rapaport, S.; Guest, G. M., and Wing, M.: Size, Hemoglobin Content, and Acid-Soluble Phosphorus of Rabbits with Phenylhydrazine-Induced Reticulocytosis, *Proc. Soc. Exper. Biol. & Med.* 57:344, 1944.

18. Allison, A. C., and Burn, G. P.: Enzyme Activity as a Function of Age in the Human Erythrocyte, *Brit. J. Haemat.* 1:291, 1955.

19. Troup, S. B., and Young, L. E.: Personal communication.

20. Pritchard, J. A.: Erythrocyte Age and Cholinesterase Activity, *Am. J. Physiol.* 158:72, 1949.

21. Sabine, J. C.: The Cholinesterase of Erythrocytes in Anemia, *Blood* 6:161, 1951.

22. Wilson, I. B.; Ginsburg, S., and Quan, C.: Molecular Complementariness as Basis for Re-activation of Alkyl Phosphate-Inhibited Enzyme, *Arch. Biochem.* 77:286, 1958.

23. Grob, D., and Johns, R. J.: Use of Oximes in the Treatment of Intoxication by Anticholinesterase Compounds in Normal Subjects, *Am. J. Med.* 24:497, 1958.

24. Wagley, P. F., and Rumsfeld, J. A.: A Clinical Note on Marchiafava-Micheli Disease, *A.M.A. Arch. Int. Med.* 101:300, 1958.

25. Crosby, W. H.: Paroxysmal Nocturnal Hemoglobinuria: Relation of the Clinical Manifestations to Underlying Pathogenic Mechanisms, *Blood* 8:769, 1953.

26. Dameshek, W.: Paroxysmal Nocturnal Hemoglobinuria: (Marchiafava-Micheli Syndrome), *Bull. New England M. Center* 4:224, 1942.

27. Bergmann, F., and Shimoni, A.: The Changes in the Nerve Membrane and the Role of Cholinesterase in the Conductive Process, *Biochim. et biophys. acta* 10:49, 1953.

28. Cheli, R., and Dianzani, M. U.: Absorption of PR8 Influenza Virus on the Red Cells of

Guinea Pigs Treated with Haemolytic Serum, *Acta haemat.* 14:15, 1955.

29. Cheli, R., and Dianzani, M. U.: The Erythrocyte Acetylcholinesterase in Experimental Haemolytic Anaemia in Guinea Pigs, *Acta haemat.* 16:37, 1956.

30. Hershey, A. D.: Bacteriophages as Genetic and Biochemical Systems, *Advances Virus Res.* 4:25, 1957.

31. Hestrin, S.: Acylation Reactions Mediated by Purified Acetylcholine Esterase: II. *Biochim. et biophys. acta* 4:310, 1950.

32. Greig, M. E., and Holland, W. C.: Studies on the Permeability of Erythrocytes: I. Relationship Between Cholinesterase Activity and Permeability of Dog Erythrocytes, *Arch. Biochem.* 23: 370, 1949.

33. Holland, W. C., and Greig, M. E.: Studies on Permeability: II. The Effect of Acetylcholine and Physostigmine on the Permeability of Potassium of Dog Erythrocytes, *Arch. Biochem.* 26: 151, 1950.

34. Lindvig, P. E.; Greig, M. E., and Peterson, S. W.: Studies on Permeability: V. The Effects of Acetylcholine and Physostigmine on the Permeability of Human Erythrocytes to Sodium and Potassium, *Arch. Biochem.* 30:241, 1951.

35. Holland, W. C., and Graham, J. H.: Factors Affecting the Rates of Hemolysis of Mammalian Erythrocytes in Isotonic Solutions of Choline and Certain Choline Esters, *Am. J. Physiol.* 183:538, 1955.

36. Auditore, J. V., and Hartmann, R. C.: Hemolysis of PNH Erythrocytes in Isotonic Solutions of Choline Esters, *J. Appl. Physiol.* 14:589, 1959.

37. Torp, H. E.: Has the Enzyme System Acetylcholine-Cholinesterase Any Significance for Physiological Hemolysis in the Spleen? *Scandinav. J. Clin. & Lab. Invest.* 8:84, 1956.

38. Wagley, P. F., and Hickey, M. D.: Susceptibility of Red Cells and Serum Factor in the Mechanism of Hemolysis in Paroxysmal Nocturnal Hemoglobinuria, *J. Clin. Invest.* 27:559, 1948.

39. Petty, C. S.: Organic Phosphate Insecticide Poisoning: Residual Effects in 2 Cases, *Am. J. Med.* 24:467, 1958.

40. Namba, T., and Hiraki, K.: PAM (Pyridine-2-Aldoxime Methiodide) Therapy for Alkylphosphate Poisoning, *J.A.M.A.* 166:1834, 1958.

41. De Sandre, G., and Ghiotto, G.: An Enzymic Disorder in the Erythrocytes of Paroxysmal Nocturnal Haemoglobinuria: A Deficiency in Acetylcholinesterase Activity, *Brit. J. Haemat.* 6:39, 1960.

42. Barry, R. M.: The Phospholipid Distribution in the Erythrocyte in Paroxysmal Nocturnal Haemoglobinuria, *Brit. J. Haemat.* 5:212, 1959.

Ischemic Infarction and Swelling in the Rat Brain

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Cerebral infarction in man may produce brain swelling and increased intracranial pressure.^{1,2} This paper concerns the use of bilateral carotid ligation in rats to provide an experimental model for the study of massive cerebral infarction and swelling. Some of the factors affecting mortality following bilateral carotid ligation have been elucidated, as well as the anatomical signs of brain swelling following massive infarction and the distribution of lesions in surviving rats.

Methods

Female albino rats, weighing 100-260 gm., of a Wistar-derived strain were used. Both common carotid arteries were doubly ligated and cut (with minimal trauma to accompanying nerves) in groups of 10 or 12 rats under pentobarbital or ether anesthesia. Rectal temperatures were measured immediately after operation and occasionally thereafter. The rats were housed individually. The skulls of rats that died or were killed in moribund state were fixed in 10% formalin after opening the anterior ends, and decalcified in 5% nitric acid. A frontal cut was made 2 mm. anterior to the parietal-interparietal suture, passing just behind the corpus callosum. This left the posterior tips of the cerebral hemispheres lying free in the concavities of the two halves of the tentorium. The tentorial region was inspected by lifting out one or both hemispheric tips, which were then replaced and the frontal slices completed. The slices, including almost the entire skull and brain, were embedded in paraffin and sectioned; in many cases serial sections were cut in the tentorial region. (Normal rats were treated in identical fashion, including serial sections, for comparison.) Surviving rats were killed with chloroform; usually brains of these rats were removed from the skulls before fixation. Sections were stained by hematoxylin and eosin and by Luxol fast blue-periodic acid-Schiff-hematoxylin.

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Results

Mortality.—Bilateral carotid ligation was performed on a total of 200 rats, of which 128 died during the first 24-hour period, 11 died the day after surgery, and 16 died between 2 and 6 days after surgery. Forty-five rats survived and were killed between two and six days after surgery.

Mortality varied greatly from day to day in the early experiments. The impression that variation in mortality depended on the weather was investigated by controlled experiments on 46 rats performed on four warm, humid days. In each of the four experiments, the variation in weight among the rats was never more than 22 gm. The rats were selected at random, and bilateral ligation was performed serially. Immediately after ligation, the rats were removed to an air-conditioned room or left in the laboratory, in alternating order. Only 2 out of 23 rats survived 24 hours in the warm, humid environment of the laboratory, while 13 out of 23 rats survived in the air-conditioned room (Table 1). In addition, the duration of life among rats that died was prolonged

TABLE 1.—Influence of Air Conditioning on Survival of Rats Following Bilateral Carotid Ligation Under Pentobarbital Anesthesia

Experiment	With Air Conditioning *		Without Air Conditioning *	
	Room Temp., C	Survivals	Room Temp., C	Survivals
1	24	2/5	29	0/5
2	25	3/6	32	1/6
3	25	3/6	33.5	0/6
4	25	5/6	30	1/6
Totals		13/23		2/23

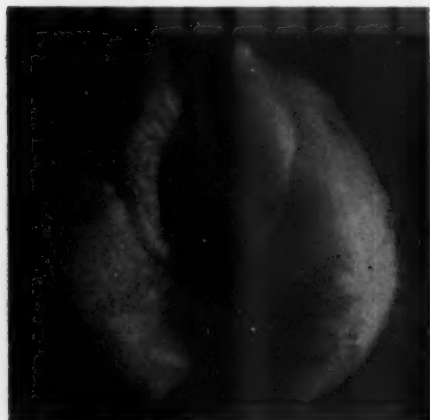
* Temperatures recorded are average afternoon readings on the day of ligation. Humidity was also decreased in the air-conditioned room, but no measurements were made.

ISCHEMIC INFARCTION IN RAT BRAIN

an average of two hours by air conditioning. Rectal temperatures taken immediately after surgery varied from 33.6-38.0 C, but there was no correlation between this value and the outcome. The temperatures of almost all the rats in the air-conditioned room decreased 1-4 C within a few hours after operation. In contrast, the temperature of almost every rat left in the warm laboratory showed no change or an increase of up to 2.6 C; in other experiments elevations of up to 5.4 C have been observed. In an additional experiment on 12 rats performed entirely with an air-conditioned environment the survival rate was not improved further by injection of 25,000 units of procaine penicillin G (three survivors out of six) as compared with alternated sham-injected controls (four out of six).

The effects on survival of the anesthetic agent and the size of the rat were investigated in experiments on 44 rats conducted entirely without air conditioning. Alternation of the anesthetic agent resulted in survival of 1 out of 10 ligated under ether and 4 out of 10 under pentobarbital anesthesia; it is uncertain whether this difference is significant. Alternation of size of rat resulted in survival of 3 out of 12 large (230 gm.) rats and 3 out of 12 small (100 gm.) rats.

Fig. 1.—Tentorial pressure grooves on posterior aspect of cerebral hemispheres. Scale in millimeters.



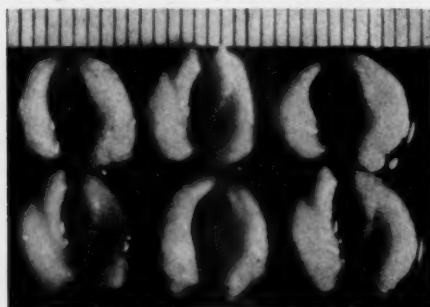
Levine-Klein

The brains of all rats from the above experiments, plus an additional 98 rats subjected to bilateral ligation, were studied pathologically. The latter group, most of which were ligated without benefit of air conditioning, included preliminary studies of the influence on survival of urea, chlorpromazine, and induced hypothermia. We have not, so far, been able to improve the survival rate with these methods, although hypothermia prolonged life.

Pathologic Findings.—Most of the rats that died or became moribund within 48 hours after bilateral carotid ligation showed cerebral infarction and swelling. Necrosis usually involved most of both hemispheres, diencephalon, and tectum of the midbrain, but in some cases it was unilateral. Necrosis was detectable and distinguishable from postmortem autolysis in rats that died as early as three to four hours after ligation: early changes were perivenous in location. There were neuronal shrinkage, nuclear pyknosis or pallor, widening of perineuronal and pericapillary spaces, rarefaction and vacuolation of intercellular tissue, and vascular congestion. The anterior pituitary often showed necrosis, which varied from focal to subtotal in extent and was characterized by nuclear shrinkage and pyknosis and by cytoplasmic eosinophilia and vacuolation.

Forebrain swelling was detected grossly by the presence of curved grooves on the posterior aspects of the cerebral hemi-

Fig. 2.—Cerebral hemispheres (posterior tips) with tentorial pressure grooves (top center, bottom left and right), compared with normals (top left and right, bottom center). Scale in millimeters.



77/545



Fig. 3.—Groove and herniation around edge of dural tentorium. Midbrain is on right. Hematoxylin and eosin stain; $\times 30$.

spheres (Fig. 1), which appeared in rats that lived at least 8-12 hours. Some normal brains showed slight indentations, but this was clearly distinguishable from the deeper, sharper grooves due to pressure of the swollen forebrain against the rigid bony and dural tentorium (Fig. 2). In some cases, the tentorial incisura around the midbrain was widened, thereby exposing a portion of cerebellum to view from the front. Histologic serial sections of the tentorial area often showed the groove. The sections

Fig. 5.—Herniation of cerebral tissue between dural tentorium (left) and midbrain (right). The posterior cerebral artery passes between the herniated tissue and the midbrain. Hematoxylin and eosin; $\times 125$.

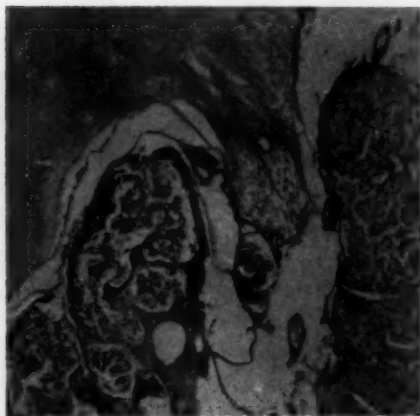
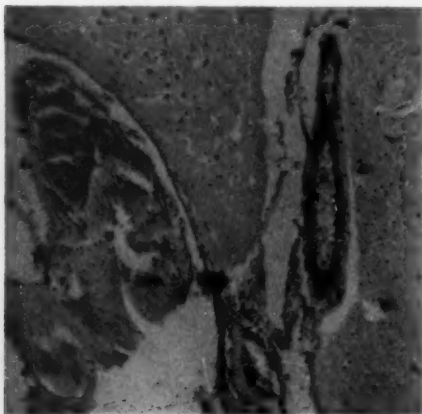


Fig. 4.—Herniation of cerebral tissue between bony tentorium (left) and midbrain (right). The herniated tissue shows severer necrosis and edema than contiguous areas. Hematoxylin and eosin; $\times 30$.

showed herniation of cerebral tissue around the tentorial edge (Figs. 3, 4, 5). The herniated tissue showed severer edema and necrosis than contiguous areas (Fig. 4). This proved that the herniation occurred during life, as did the presence of the groove in rats that were killed, as well as in rats that died; the groove was not produced by permitting the head of a normal rat to autolyze.

Other signs of swelling were detected more readily by histologic than by gross examination. In a few cases, the postero-

Fig. 6.—A tongue of hippocampal tissue extends between diencephalon (right upper quadrant) and trigeminal nerve (left lower quadrant) and is indented by the posterior communicating artery (arrow). Hematoxylin and eosin; $\times 30$.



inferior extremity of the hippocampus herniated downward between the ventral surface of the diencephalon and the trigeminal nerve (Fig. 6). In serial sections, this appeared to be a continuation of the transtentorial herniation described above, and, in fact, the trigeminal nerve was partly covered by a thin bony prolongation of the bony tentorium. The herniated hippocampal tissue was usually indented by the posterior communicating artery (Fig. 6). Indentations of cerebral substance by arteries were noted in other areas. Most prominent was indentation of the pons by the anterior end of the basilar artery. In a few cases, the indentation was so deep that the artery was almost completely surrounded by pontine tissue (Fig. 7).



Fig. 7.—Anterior end of the basilar artery almost completely surrounded by pontine tissue. Hematoxylin and eosin; $\times 125$.

In frontal sections of the brains of normal rats, the configuration of the dorso-medial cortex of each hemisphere consisted of a smooth curve, with convexity facing the superior longitudinal sinus (Fig. 8). In rats with bilateral swelling, the configuration in this area was that of a right angle; thus the medial cortices of the two hemispheres were in close apposition, instead of diverging gradually near the superior longitudinal sinus (Fig. 9). This change often appeared early after ligation (three to four hours) and was prominent in the posterior half of the hemispheres. In rats with unilateral swelling, the configuration was an

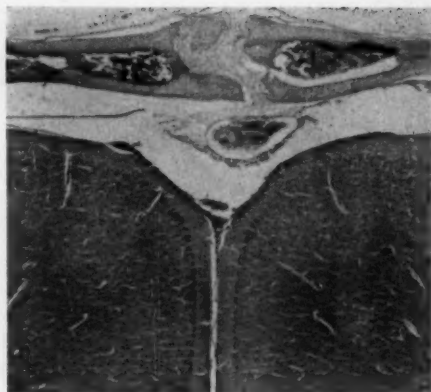


Fig. 8.—In a normal rat, the configuration of the dorsomedial cortex of each hemisphere consists of a smooth curve with convexity facing the superior longitudinal sinus. Compare Figure 9. Hematoxylin and eosin, $\times 30$.

obtuse angle on one side and an acute angle on the other side, with the medial cortex of the swollen hemisphere bulging into the normal hemisphere.

At the posterior ends of the hemispheres of normal rats, the medial surfaces were separated by the pineal, which had a round or oval cross section with dorsal indentation by the overlying venous sinus (Fig. 10). In rats with swelling of the hemispheres, the pineal was compressed into a

Fig. 9.—In a rat with bilateral forebrain swelling, the configuration of the dorsomedial cortex of each hemisphere is that of a right angle. The medial cortices of the two hemispheres are in close apposition, instead of diverging gradually near the superior longitudinal sinus. Hematoxylin and eosin; $\times 30$.

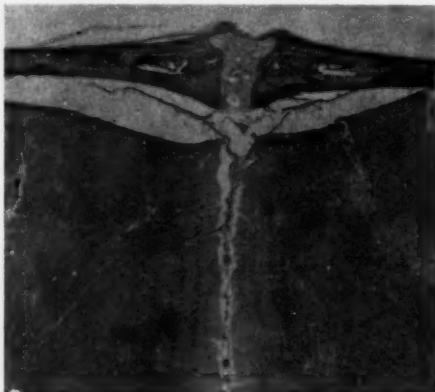


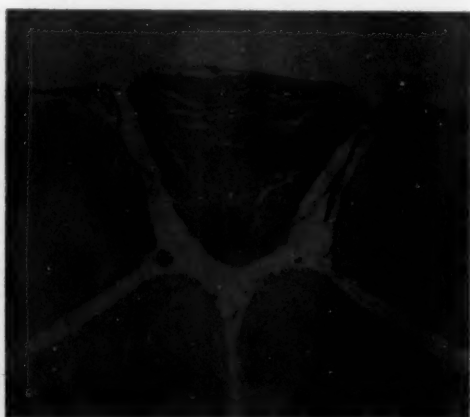


Fig. 10.—In a normal rat, the pineal has a rounded cross section, indented by the overlying venous sinus. Compare Figure 11. Hematoxylin and eosin; $\times 30$.

triangular shape with apex downward (Fig. 11). The tectum of the midbrain, just below the pineal, was compressed and narrowed by the same mechanism. The pituitary was flattened, and the internal carotid artery grooves were deeper than normal.

necrosis (Fig. 12) without predilection for a particular lamina, but occasionally there was laminar or isolated neuronal necrosis. Only a few rats showed massive cortical destruction; these were invariably unilateral, probably because rats with bilateral

Fig. 11.—In a rat with forebrain swelling, the pineal is compressed into a triangular shape between the two swollen hemispheres. The tectum of the midbrain (below pineal) appears compressed also. Hematoxylin and eosin; $\times 30$.



Focal Lesions.—The incidence and distribution of focal ischemic lesions were studied in rats that lived two to six days after bilateral carotid ligation. During this period 16 rats died (5 had brain lesions) and 45 rats were killed (19 had brain lesions). The areas involved in the 24 rats with lesions are indicated in Table 2. Cerebral cortex and corpus striatum were involved in most instances. The cortex showed small, round, unilateral or bilateral foci of

TABLE 2.—Distribution of Focal Brain Lesions Found in Twenty-Four Out of Sixty-One Rats That Survived Two to Six Days Following Bilateral Common Carotid Artery Ligation

	No. of Rats with Lesions in Stated Region
Cerebral cortex	20
Corpus striatum (gray matter only)	11
Corpus striatum (gray and white matter)	7
Hippocampus	6
Corpus callosum and callosal radiation	8

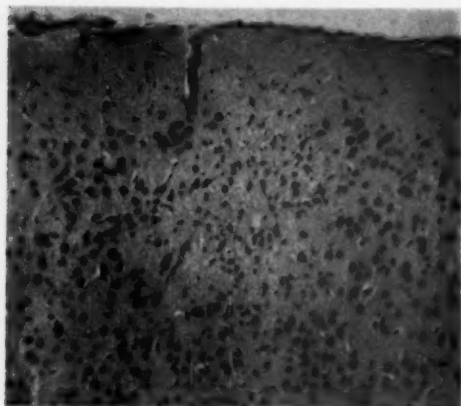


Fig. 12.—Focal ischemic necrosis in cerebral cortex. The neurons are shrunken and the intercellular tissue vacuolated. Hematoxylin and eosin; $\times 125$.

lesions of comparable severity died early. The corpus striatum, in fact most of the hemisphere, was involved in these few massive unilateral lesions. More frequently, involvement of the striatum consisted of

irregularly distributed, unilateral or bilateral, patchy areas of necrosis of gray matter (Fig. 13). The white matter was involved also in a few rats, but no selective lesions of white matter were observed. One

Fig. 13.—Ischemic necrosis of gray matter of corpus striatum. The shrunken necrotic neurons on the left contrast with the single intact neuron on the right. Hematoxylin and eosin; $\times 500$.

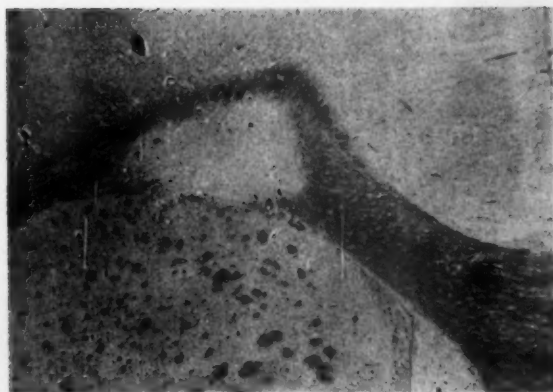
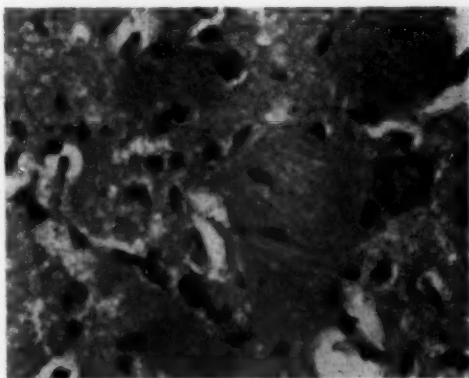


Fig. 14.—Ischemic necrosis of white matter of callosal radiation in a rat that had relatively minor damage to cortex and striatum. Luxol fast blue-periodic acid-Schiff-hematoxylin; $\times 30$.

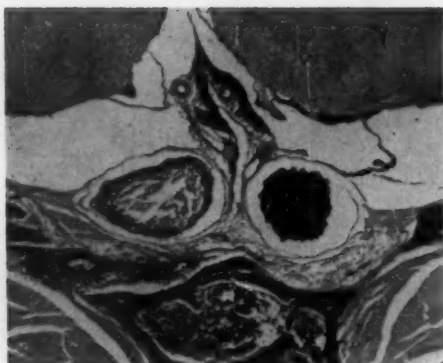


Fig. 15.—Ischemic necrosis of one optic nerve in a rat that had severe unilateral infarction. Luxol fast blue-periodic acid-Schiff-hematoxylin; $\times 30$.

rat showed bilateral symmetric necrosis of gray and white matter of the striatum, of the type observed commonly in histotoxic anoxia (cyanide, azide).

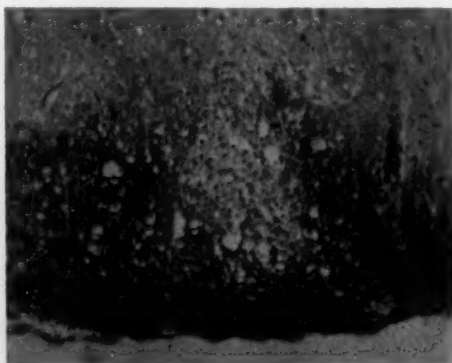
Hippocampal lesions were relatively few. There was severe necrosis only in the cases of massive unilateral damage. The others showed mild injury with necrosis of scattered neurons. Lesions of the corpus callosum and callosal radiation were commoner than expected. A few occurred in rats with massive hemispheric necrosis, but others appeared as plaques of necrosis (Fig. 14) in rats that had only mild or moderate damage to cortex and striatum. In a few rats with extensive destruction, there was involvement of optic nerve (Fig. 15) and chiasm, thalamus, hypothalamus, and lateral olfactory tract (Fig. 16).

Fig. 16.—Focal ischemic necrosis in lateral olfactory tract. Same rat as Figure 15. Luxol fast blue-periodic acid-Schiff-hematoxylin; $\times 125$.

Comment

Mortality.—Chang and Liu³ reported that all of 10 rats (size and sex not stated) died within 24 hours after simultaneous bilateral carotid ligation.* In contrast, Jilek⁴ reported only 40% mortality within 24 hours in rats weighing 140-300 gm. and 71% mortality in rats less than 140 gm. in weight but more than 32 days of age. In addition to probable differences in strain, sex, size, diet, and surgical technique, our results point to atmospheric conditions as a possible explanation for this discrepancy (Table 1). Jilek kept his animal room at 26 C, and his mortality rate, of 40%, is similar to our rate of 43% obtained in air-conditioned quarters. Chang and Liu probably did not have air-conditioning facilities,

* Chang and Liu³ obtained survivors by allowing an interval of 2-14 days between the two ligations. Impaired maze performance was attributed to cerebral anemia, while the possible role of brain lesions was ruled out by negative histologic study of a number of brains. These conclusions are not completely admissible, because not all the brains were studied, nor was it indicated whether each brain was sectioned in entirety. Many of the lesions found in the present experiments were small and easily missed; probably the incidence of survivors without brain lesions would decrease if serial sections were made. The present paper concerns only simultaneous bilateral ligation, but similar lesions have been observed when the ligations were separated by an interval; recently we have observed an ischemic lesion after *unilateral* ligation in a single rat. Therefore brain lesions may have played a part in the impairment of maze performance observed by Chang and Liu.



and their rate, of 100%, is close to our rate of 91%, obtained on warm days without benefit of air conditioning. Our data suggest that the beneficial effect of air conditioning and the adverse effect of warm environment were mediated by fall or rise in body temperature. It is not certain whether a disturbance of thermoregulation due to ischemia or necrosis of the hypothalamic centers was involved. A warm, humid environment interferes with the normal process of heat loss, and this causes disturbance of many body functions.⁵ In addition, a warm environment causes a decrease of blood pressure,^{5,6} and this, in turn, may cause failure of the collateral circulation,⁷ with disastrous consequences. On the other hand, air conditioning facilitates normal heat loss, and any resulting hypothermia increases the resistance of the brain to anoxia.[†] It has been shown that air conditioning has beneficial effects on many patients with heart disease and other conditions,⁸ and the present results suggest that patients with cerebral infarction may receive similar benefit.

Brain Swelling.—The signs of brain swelling that have been described are due to the enlarged forebrain filling out all available space in front of the tentorium, displacing cerebrospinal fluid, compressing venous sinuses, and pushing into the posterior fossa. Certain of the signs are exactly comparable to the tentorial pressure groove, uncal herniation, and flattened gyri observed in humans with supratentorial swelling or space-occupying lesions. However, brain-stem hemorrhages and aqueduct obstruction were not observed in the rats.

Lindenberg⁹ has pointed out that increased intracranial pressure can lead to compression of various arteries and consequent secondary ischemic lesions in humans. Wheatley¹⁰ showed that forebrain swelling with resultant vascular obstruction at the level of the tentorium was responsible for

death in cats given large doses of sodium cyanide. Although the posterior cerebral artery of the rat passed between the mid-brain and transtentorial herniated cerebral tissue (Fig. 5), we have no proof of compression; the occurrence of arterial grooves in the brain (Figs. 6, 7) suggested that the brain rather than the arteries was compressed. Be that as it may, brain swelling certainly can compress veins, capillaries, and arterioles. The resultant interference with collateral circulation, verified by direct observation through a skull window by Meyer,¹¹ can lead to a vicious circle of infarction and swelling, terminating in death.^{2,11} In the present experiments it was noted that almost all the rats that escaped massive infarction and early death suffered focal lesions of relatively slight or moderate extent or had no lesions at all. Only four rats with fairly extensive necrosis survived 48 hours, and the lesions were unilateral in all. The paucity of such instances may be due to a vicious circle if we assume that infarctions beyond a certain threshold of severity, especially if bilateral, are associated with so much swelling as to produce more infarction, and so on.

Focal Lesions.—It is remarkable that bilateral carotid ligation, performed with standardized technique, produced results varying from nil (37 rats) to massive fatal infarction (139 rats). Probably, this variation is due to individual differences in collateral circulation. The intermediate group, of 24 rats, that survived two days or more with focal ischemic brain lesions is of especial interest, because the areas involved may represent the regions of the normal brain with the most tenuous balance between blood supply and metabolic demand. Unfortunately, the results with carotid-ligated rats cannot be extrapolated back to the normal, intact animal because the experimental procedure has not merely reduced the flow in the carotid system, but has increased the importance of the collateral circulation, so that the latter becomes the limiting factor which determines the outcome. With this reservation in mind, it is nevertheless of in-

[†] The factor of dehydration in rats kept in a warm environment might be expected to reduce brain edema; yet these animals showed higher mortality.

terest to note the preponderance of lesions in cerebral cortex and corpus striatum (Table 2), which is in accord with the high rates of metabolism recorded for these regions.¹² On the other hand, the hippocampus, usually regarded as highly vulnerable to anoxia (due to special features of its vasculature¹³), showed fewer lesions. It is difficult to explain this or to correlate it with Hicks' observation¹⁴ that the rat hippocampus was spared after asphyxia in nitrogen, and our previous observation¹⁵ that it was the most vulnerable region when anoxic anoxia (nitrous oxide or nitrogen) and ischemia (unilateral carotid ligation) were combined.

The lesions of corpus callosum and callosal radiation were more numerous than might be expected, in view of the low metabolic demand of white matter. The lesions were single or multiple foci of necrosis (Fig. 14) which showed no preference for any special part of these structures and which never reproduced the characteristic symmetrical midline lesion of cyanide intoxication.^{14,16} While not contravening the premise that cyanide affects white matter because of the paucity of cytochrome oxidase,¹⁷ the present results suggest the possibility that the exact localization of cyanide lesions in corpus callosum and callosal radiation may be due, in part, to relatively poor blood supply of these white matter areas. This conforms to Hicks' conclusion¹⁷ that the "vulnerability of various parts of the nervous system to metabolic injury must be considered to be compounded of several factors."

Applications.—Bilateral carotid ligation in rats may prove to be a useful method for evaluating therapy for brain infarction and swelling, providing alternate controls and attention to climatic factors are incorporated into the experimental design. Cold injury has been used in therapeutic experiments on larger animals¹⁸; the lesions differed in being hemorrhagic. Possible advantages of the method presented here include speed and simplicity of the operative procedure, low

cost of rats as test animals, and easy recognition of swelling by gross or microscopic criteria.

Summary

Bilateral carotid ligation in rats provided an experimental model for the study of cerebral infarction and swelling. The mortality depended on the weather and was greatly reduced by use of air conditioning on warm days. Rats that died of infarction showed cerebral swelling, manifested by tentorial pressure grooves, arterial indentations, close apposition of medial cortices of the two hemispheres, and pineal and mid-brain compression. There was evidence of a vicious-circle mechanism, involving infarction and swelling. Less than half of the surviving rats showed lesions; these involved cerebral cortex and corpus striatum, and, less frequently, hippocampus, corpus callosum, callosal radiation, and other areas. The patchy involvement of white matter suggested that vascular factors may play a role in the localization of leukoencephalopathy due to cyanide.

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REFERENCES

1. Shaw, C. M.; Alvord, E. C., Jr., and Berry, R. E.: Swelling of the Brain Following Ischemic Infarction with Arterial Occlusion, *A.M.A. Arch. Neurol.* 1:161-177, 1959.
2. Hicks, S. P., and Warren, S.: Infarction of the Brain Without Thrombosis: An Analysis of 100 Cases with Autopsy, *A.M.A. Arch. Path.* 52: 403-412, 1951.
3. Chang, H. C., and Liu, S. Y.: The Influence of the Ligation of the 2 Common Carotid Arteries on Maze Performance by the White Rat, *J. Comp. & Physiol. Psychol.* 8:71-74, 1928.
4. Jilek, L.: Reaction of the Organism to Ischaemia of the Brain During Ontogenesis: II. The Development of Functional Changes in the Central Nervous System Following Ligation of the Carotids During Postnatal Life in Rats, *Physiol. Bohem.* 7:282-291, 1958.
5. Mills, C. A.: *Medical Climatology: Climatic and Weather Influences in Health and Disease*, Springfield, Ill., Charles C Thomas, Publisher, 1939.

ISCHEMIC INFARCTION IN RAT BRAIN

6. McDowall, R. J. S.: The Control of the Circulation of the Blood, London, William Dawson & Sons, Ltd., 1956.
7. Denny-Brown, D., and Meyer, J. S.: The Cerebral Collateral Circulation: 2. Production of Cerebral Infarction by Ischemic Anoxia and Its Reversibility in Early Stages, *Neurology* 7:567-579, 1957.
8. Burch, G. E., and DePasquale, N.: Influence of Air Conditioning on Hospitalized Patients, *J.A.M.A.* 170:160-163, 1959.
9. Lindenberg, R.: Compression of Brain Arteries as Pathogenetic Factor for Tissue Necroses and Their Areas of Predilection, *J. Neuropath. & Exper. Neurol.* 14:223-243, 1955.
10. Wheatley, M. D.: Mechanism of Cerebral Death with Doses of NaCN from Which the Heart Recovers, *J. Neuropath. & Exper. Neurol.* 6:295-298, 1947.
11. Meyer, J. S.: Localized Changes in Properties of the Blood and Effects of Anticoagulant Drugs in Experimental Cerebral Infarction, *New England J. Med.* 258:151-159, 1958.
12. Himwich, H. E.: *Brain Metabolism and Cerebral Disorders*, Baltimore, The Williams & Wilkins Company, 1951.
13. Scharrer, E.: The Blood Vessels of the Nervous Tissue, *Quart. Rev. Biol.* 19:308-318, 1944.
14. Hicks, S. P.: Brain Metabolism in Vivo: I. The Distribution of Lesions Caused by Cyanide Poisoning, Insulin Hypoglycemia, Asphyxia in Nitrogen and Fluoracetate Poisoning in Rats, *Arch. Path.* 49:111-137, 1950.
15. Levine, S.: Anoxic-Ischemic Encephalopathy in Rats, *Am. J. Path.* 36:1-17, 1960.
16. Levine, S., and Stypulkowski, W.: Experimental Cyanide Encephalopathy, *A.M.A. Arch. Path.* 67:306-323, 1959.
17. Hicks, S. P.: Brain Metabolism in Vivo: II. The Distribution of Lesions Caused by Azide, Malononitrile, Plasmocid and Dinitrophenol Poisoning in Rats, *Arch. Path.* 50:545-561, 1950.
18. Raimondi, A. J.; Clasen, R. A.; Beattie, E. J., and Taylor, C. B.: The Effect of Hypothermia and Steroid Therapy on Experimental Cerebral Injury, *Surg. Gynec. & Obst.* 108:333-338, 1959.

"Bronchiolar Emphysema of the Lungs"

Report of a Case

H. T. RAVINES, M.D., Arequipa, Peru

In 1957 Siebert and Fisher¹ reported two cases of unusual pulmonary disease and reviewed 10 similar cases from the literature. On the basis of what they considered the principal site of the morphologic alteration, these authors proposed the name "bronchiolar emphysema" to replace the terms cystic cirrhosis of the lungs, or muscular cirrhosis of the lungs.² The morphologic characteristics of this unusual entity had been previously described by von Stössel,³ Calma,⁴ and Rubenstein, Gutstein, and Lepow.⁵ As the condition is rare, it is worth while to report an additional case which shows many of the characteristic features of the cases previously described.

Report of Case

The patient, a 78-year-old white woman, was admitted to the University of Kansas Medical Center five days before death, complaining mainly of severe dyspnea and generalized weakness. Her past history revealed that for the past four years she had had progressive dyspnea on exertion and a chronic productive cough. She had been treated with digitalis without any improvement. On the day prior to her hospitalization she had had a small hemoptysis.

On physical examination she was found to be intensely dyspneic. Her blood pressure was 110/70 mm. Hg; pulse 110 per minute and regular. Auscultation of the chest revealed moist rales in the lower half of both lungs. Roentgenologic studies of the chest revealed a diffuse patchy infiltration fanning out from the hilus into both lung fields. The heart showed sinus tachycardia. The liver was enlarged 2 fingerbreadths below the right costal margin.

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Electrocardiographic studies suggested myocardial ischemia. Hematocrit was reported as 55%; hemoglobin was 15.6 gm. %. The white blood cell count was 11,000 per cubic millimeter. The blood carbon dioxide was 22.2 mEq. per liter; sodium 135 mEq. per liter, and potassium 3.8 mEq. per liter.

Her hospital stay was characterized by severe dyspnea, cyanosis, and mental confusion. Her condition deteriorated rapidly, and she eventually died in respiratory failure.

Gross Findings

At autopsy the thorax was seen to be slightly barrel-shaped. The salient pathological features were confined to the heart and lungs. The lungs weighed 1,400 gm. There were fibrous adhesions over both upper lobes. On external examination

Fig. 1.—The lung surfaces are markedly roughened by projecting nodules and show an appearance similar to that seen in hepatic cirrhosis.



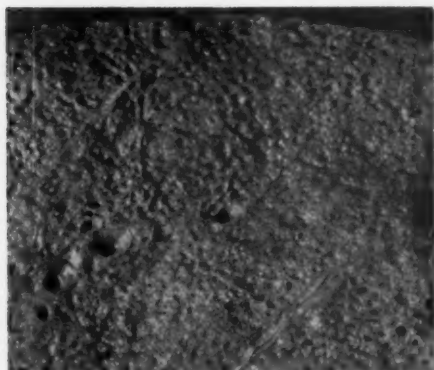


Fig. 2.—Honeycomb lung with cysts surrounded by fibrous tissue. To lower right there is a relatively normal bronchus.

the entire pleural surfaces of the lungs showed a bosselated appearance, similar to that seen in a liver with portal cirrhosis (Fig. 1). At the periphery of the lower lobes a few emphysematous blebs, averaging 0.7 cm. in size, were present. On sections both lungs showed a honeycombed appearance (Fig. 2); numerous cysts containing air, ranging from 0.15 to 0.5 cm. in diameter, throughout the parenchyma were separated by thick fibrotic walls. There were scattered areas of lung tissue which were brownish-red in color and appeared consolidated. No obviously normal lung tissue was present. The large bronchi showed no significant changes.

The smaller bronchi of the third order showed mild diffuse dilatation. Their mucosa was smooth, and grayish in color, with faint transverse plicae.

Occasional areas of mucosal ulceration were noted. No direct continuity could be traced on gross examination between the bronchi and the cysts.

In addition, the right apex contained two areas of fibrous scarring, averaging 1.3 cm. each. A calcified nodule, measuring 1.5 cm. in diameter, was found near the hilus of the left upper lobe. A solitary 0.5 cm. rubbery nodule was seen in the middle of the parenchyma of the right upper lobe. The tissues surrounding the nodule did not show scarring.

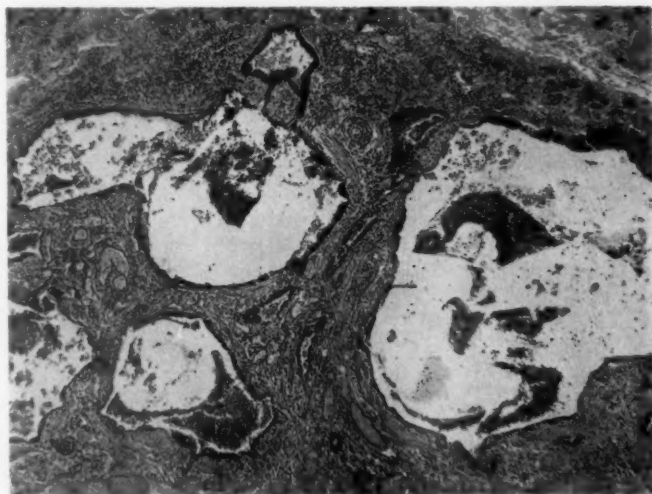
The pulmonary artery disclosed a few small atheromatous plaques, but did not appear dilated. The branches of pulmonary arteries showed marked atherosclerosis. The heart weighed 390 gm.; it showed marked hypertrophy and dilatation of the right ventricle, the right atrium, and the infundibulum of the pulmonary artery.

No significant lesion was noted in the remaining organs apart from passive congestion.

Microscopic Observations

Microscopic sections of both lungs showed similar diffuse structural changes, tending to be most marked toward the periphery. In sections of many areas normal lung tissue was not observed, the parenchyma of the lung being replaced by dense fibrous tissue in which numerous cysts of varying size and shape were present. These cysts were lined by columnar, cuboidal, and flattened epithelium, which had ulcerated in many areas. In other areas squamous metaplasia was noted. The walls of the cysts consisted of vascular fibrous tissue, which also had

Fig. 3.—Note well-vascularized fibrous tissue forming the walls of cystic bronchioli and replacing pulmonary parenchyma. The tissue shows bundles of hypertrophied smooth muscle within the cyst walls and a diffuse infiltrating by inflammatory cells. The cysts contain an inflammatory exudate. Hematoxylin and eosin stain; reduced to 88% of mag. $\times 40$.



replaced the normal parenchyma (Fig. 3). This tissue was composed of interlacing bands of collagen and reticulin and fragmented elastic fibers, and was extremely vascular, containing numerous small blood channels lined by thin endothelial cells. The tissue showed a diffuse infiltration of polymorphonuclear leukocytes, lymphocytes, and mononuclear cells, extending throughout the lung. The most interesting feature, however, was presence of numerous irregular bundles of hypertrophied smooth muscle (Fig. 3). While in many areas these appear related to the cyst walls, in others they were scattered haphazardly through the tissue.

Although the above changes predominated, in some areas foci of relatively normal parenchyma remained, the alveolar walls showing slight to moderate fibrosis with alveolar and bronchiolar dilatation. Many of these alveoli had a cuboidal lining and contained varying numbers of macrophages.

In a few places the acute inflammation predominated, with fibrin membranes lining the alveolar walls. In a few areas fibroblastic organization of the intra-alveolar exudate was found. Occasional small "tumourlets"⁶ of transitional or squamous epithelium were seen in the scarred areas of both lungs. No significant changes were seen in the bronchi. There was moderate thickening of the walls of the small muscular arteries and arterioles.

Comment

The outstanding features of this case were (a) damage of the pulmonary parenchyma with dilatation of bronchioles and diffuse chronic inflammation, and (b) hypertrophy and hyperplasia of smooth muscle in peri-bronchiolar and perivascular and within fibrotic areas.

Whether the primary damage is bronchiolar or secondary to inflammation remains

obscure. Siebert and Fisher attributed the pathogenesis of this disease to congenital "respiratory hypoplasia"; nevertheless, it should be noted that 8 out of the 13 reported cases occurred in persons over 40 years of age.

The smooth muscle present probably came from bronchiolar walls. Liebow, Loring, and Felton⁷ (1953) have studied several identifiable sources of hyperplasia of smooth muscle in certain types of chronic pulmonary disease. In the present case, as in those previously reported, there was constant association between fibrosis and smooth-muscle increase and diffuse chronic inflammation (bronchiolitis).

Summary

An unusual case of chronic pulmonary disease is presented, the pathogenesis of which remains obscure.

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REFERENCES

1. Siebert, F. T., and Fisher, E. R.: Bronchiolar Emphysema: So-Called Muscular Cirrhosis of the Lungs, *Am. J. Path.* 33:1137-1161, 1957.
2. Davidsohn, C.: Über muskuläre Lungencirrhose, *Klin. Wchnschr.* 44:33-35, 1907.
3. von Stössel, E.: Über muskuläre Cirrhose der Lunge, *Beitr. klin. Tuberk.* 90:432-442, 1937.
4. Calma, I.: Cystic Emphysema of the Lungs with Interstitial Sclerosis, *Brit. J. Tuberc.* 35:40-43, 1941.
5. Rubenstein, L.; Gutstein, W. H., and Lepow, H.: Pulmonary Muscular Hyperplasia (Muscular Cirrhosis of the Lungs), *Ann. Int. Med.* 42:36-43, 1955.
6. Whitwell, F.: Tumourlets of the Lung, *J. Path. & Bact.* 70:529-541, 1955.
7. Liebow, A. A.; Loring, W. E., and Felton, W. L., II.: The Musculature of the Lungs in Chronic Pulmonary Disease, *Am. J. Path.* 29:885-911, 1953.

Occurrence of Pulmonary Edema in Sudden Asphyxial Deaths

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The term "pulmonary edema" is commonly associated with a variety of clinical conditions and often appears in the clinical descriptions of many diseases. Frequently it is found in the protocols of human autopsies covering a wide variety of deaths. The terms "lung edema" and "edema" are also used in place of pulmonary edema. Henneman,¹ in a review of the literature, listed the many diseases or deaths in which edema had been reported to appear during the progress of the disease or was observed at autopsy. These diseases included various cardiovascular, renal, and hepatic diseases, infections, central nervous system disease or damage, drowning, toxic gases of industry and warfare, and many other conditions.

Durlacher et al.² considered pulmonary congestion and edema a true postmortem phenomenon, which could develop during the first few hours of the postmortem period in rabbits killed by pentobarbital (Nembutal), magnesium sulfate, intravenous air, electrocution, rabbit punch, and the inhalation of ether. They also stated that autopsy in humans is usually delayed several hours and that pulmonary edema and congestion are almost constant findings and may result from postmortem change.

The present study was an attempt to determine which cases of sudden death might present edema, and especially whether those of an asphyxial nature did when autopsy

was performed as soon as possible after cessation of heart action. Also, whether the microscopic appearance of the lungs in sudden asphyxial deaths was similar to that of disease deaths.

Methods

Lung tissues were taken from human cases of sudden asphyxial death. In all of the 70 cases studied, the victims were apparently in good health before their accident. Most of the victims had died in a few minutes after the accident, and all were dead on arrival at the hospital. The autopsy was performed as soon as possible after death. The postmortem interval (PMI) was estimated as close as possible when not definitely known. This estimation is indicated by a question mark (?) in the Table. The cases included death by drowning, cardiac failure, brain injury, strangulation, carbon monoxide, natural gas, Freon gas, suffocation, barbiturates, electrocution, exsanguination, and the ingestion of paraldehyde, phosphorus, phenol, and mercury bichloride.

The thoracic, abdominal, and cranial organs were removed at autopsy. Gross examination was made immediately, and microscopic examination later in order to determine whether any previous lung disease was involved in the edema formation.

Two slices of tissue were taken from each lobe in order to give good representation of the lungs. The slices were cut about $\frac{1}{2}$ in. in thickness and as parallel to the diaphragmatic surface of the lungs as possible. These tissues were fixed in 10% formalin for 24 hours. Then the tissues were cut into thin rectangular blocks, about $\frac{1}{4}$ in. in thickness, placed in small metal containers, and again fixed in 10% formalin for 16 hours. After fixation, the tissues were washed in tap water for four hours, then placed in the Autotechnicon in order to be taken through the alcohols and Doxane and embedded in paraffin. Sections were cut at 6μ to 8μ and then stained with hematoxylin and eosin.

Microscopic observations were made using the $\times 25$, $\times 100$, $\times 450$, and $\times 900$ magnification. The microscopic observations are recorded in the Table. Photomicrographs were made of typical or unusual sections.

Submitted for publication June 22, 1959.

This study was submitted as part of a doctorate thesis to the Graduate School, University of Maryland.

The work was performed at the Office of the Chief Medical Examiner, State of Maryland, and the Department of Legal Medicine, University of Maryland School of Medicine and College of Physicians and Surgeons.

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Results

The 70 cases were arranged under headings of drowning, cardiac failure, brain injury, strangulation, and miscellaneous injury, which included carbon monoxide poisoning, gas asphyxias, suffocation, barbiturate narcosis, electrocution, exsanguination, and the ingestion of paraldehyde, phosphorus, phenol, and mercury chloride. The lesions to be sought were established, and arbitrary standards for degrees of severity were set up in order to help categorize the main features of the pulmonary pathology. The qualitative data are presented in the Table. The results described below were drawn from the microscopic observations. Individual cases or a group of cases were described, and then all cases were considered as a whole and generalizations drawn which include most of the cases.

Drowning.—The 15 patients had drowned either in fresh or in brackish water. Death occurred quickly, and in no case was there evidence of any previous lung disease. There was only one person with free alveolar hemorrhage, and this person had received a blow on the head before submersion. There were three cases of vascular congestion; one had a medium degree of severity, and the others each had a light degree. One of these persons was involved in an airplane crash; another jumped off a bridge, and the third was reported to have been trapped 20 ft. below the surface before drowning. The 11 other persons were involved in swimming or boating accidents without unusual circumstances. These cases yielded sections which were without hemorrhage and vascular congestion.

All 15 human lungs had sections which contained alveolar phagocytes. These phagocytes or macrophages were large cells each with small nucleus, and a great amount of cytoplasm crammed with golden-brown pigment, which was hemosiderin and anthracotic dust. Occasionally, these phagocytes were found separately in the alveoli, but they

were also scattered or in groups within the alveoli. Mostly they were free of, or in the pink-staining, stringy or granular coagulum in the alveoli taken to represent pulmonary edema (Fig. 1A, B, C, D). The lung sections of three patients contained phagocytes of a slight degree, while the lungs of nine persons had phagocytes of a medium degree and three had a heavy degree of alveolar phagocytes. The case with hemorrhage and the cases with congestion had phagocytes of a medium degree.

All 15 cases had sections which contained edema, varying in distribution and extent. Some areas had microscopic fields without edema, while other adjacent fields showed edema. In some areas the edema seemed to have involved a respiratory unit or group of respiratory units, Miller³ refers to these as primary lobules or secondary lobules. Even in individual lobules, it apparently did not fill all alveoli, air sacs, or atria. The alveoli were not always full of fixable and stainable edema fluid. In many instances the stainable edema fluid appeared at the borders of the alveolus, and not in the center (Figs. 1A and 4B). The clear areas in the alveoli represented air, as gross observations on the lungs of drowned animals have indicated that these lungs contain minute air bubbles.

The edema fluid in the drownings appeared as a pink, stringy coagulum (Fig. 1A), or as both stringy and granular (Fig. 1C), or as entirely granular (Fig. 1D). The involvement by edema ranged from the barely detectable (Fig. 4B) to the near-complete inundation of most of the alveoli (Fig. 4D). The edema was severe in only two cases. There were seven cases of edema to a medium degree and six in which the edema was light. These measurements were not quantitative; however, the edema, though moderate in degree, was fairly extensive throughout the lungs.

The peribronchial, perivascular, and subpleural lymphatics in most cases were not dilated. In the lungs of two of the victims drowned in brackish water, one of whom was known to have received a blow on the

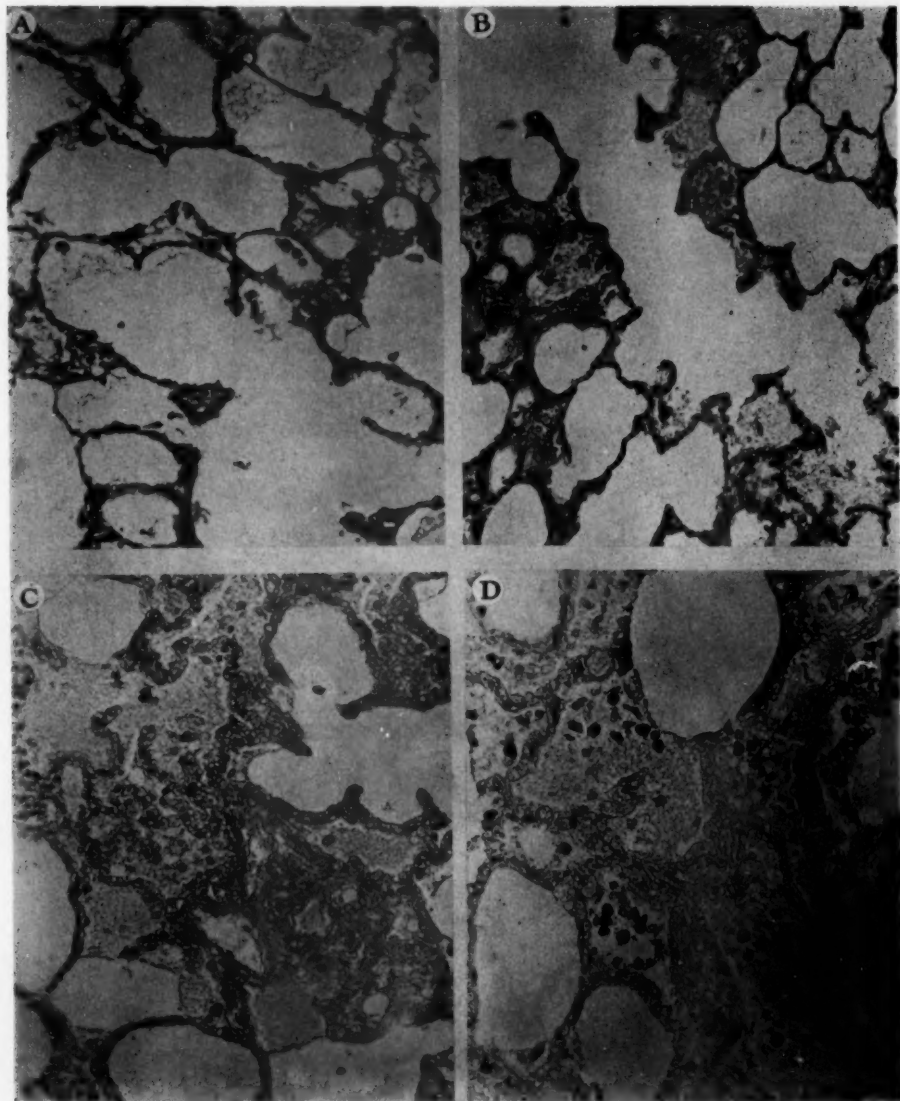


Fig. 1.—Photomicrographs of the lungs of drowned humans: formalin-fixed, paraffin-embedded, hematoxylin-and-eosin-stained; reduced to 77% of magnification $\times 115$.

A, man; contains light stringy edema fluid and a few alveolar phagocytes.

B, female child; contains light granular edema fluid and a few alveolar phagocytes.

C, man; contains medium stringy and granular edema fluid and a few alveolar phagocytes (Fig. 3D).

D, woman; contains moderate to heavy granular edema fluid and many alveolar phagocytes.

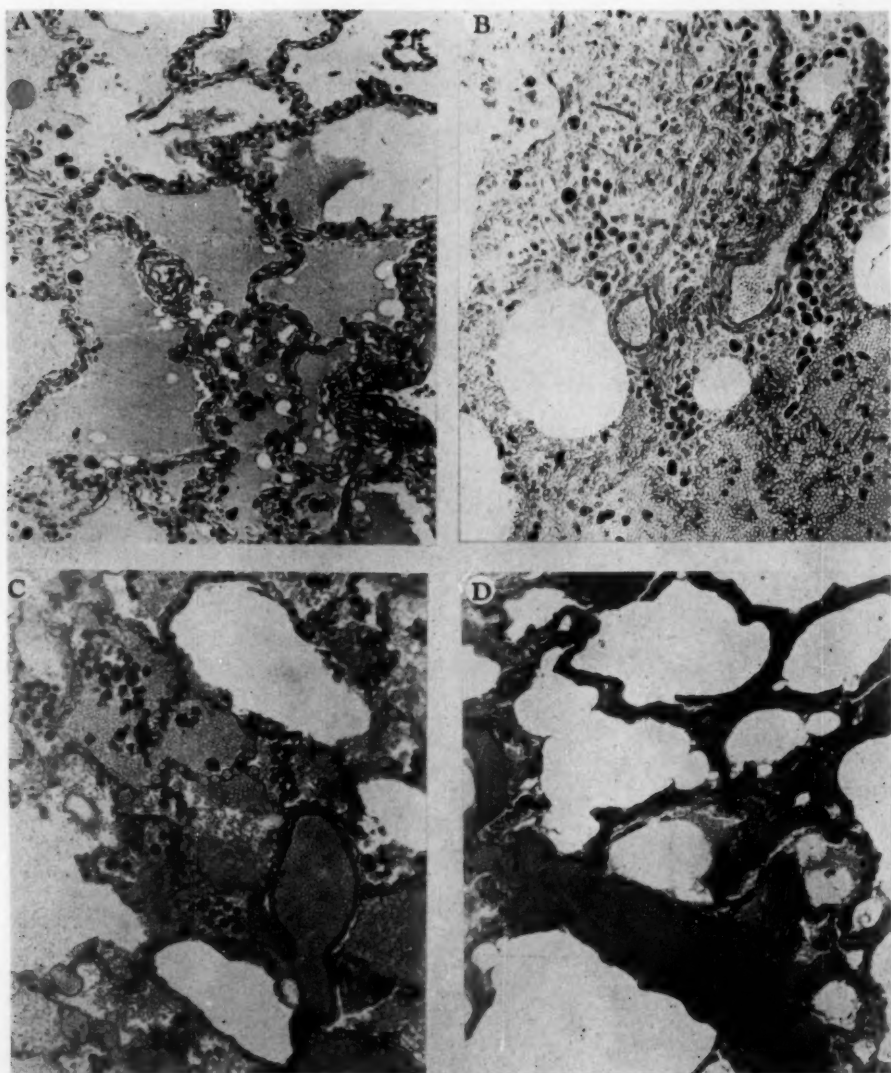


Fig. 2.—Photomicrographs of human lungs: formalin-fixed, paraffin-embedded, hematoxylin-cosin-stained; reduced to 73% of magnification $\times 115$.

A, man, cardiac failure; contains smooth, granular, and stringy edema fluid, alveolar phagocytes, and light congestion in capillaries.

B, cardiac failure; contains light stringy edema fluid in upper corners of photomicrograph, medium congestion of blood vessels, heavy hemorrhage, and many alveolar phagocytes.

C, brain injury, woman; contains stringy edema fluid, heavy vascular congestion, heavy hemorrhage, and many alveolar phagocytes.

D, strangulation, woman; contains light stringy and granular edema fluid, and heavy vascular congestion.

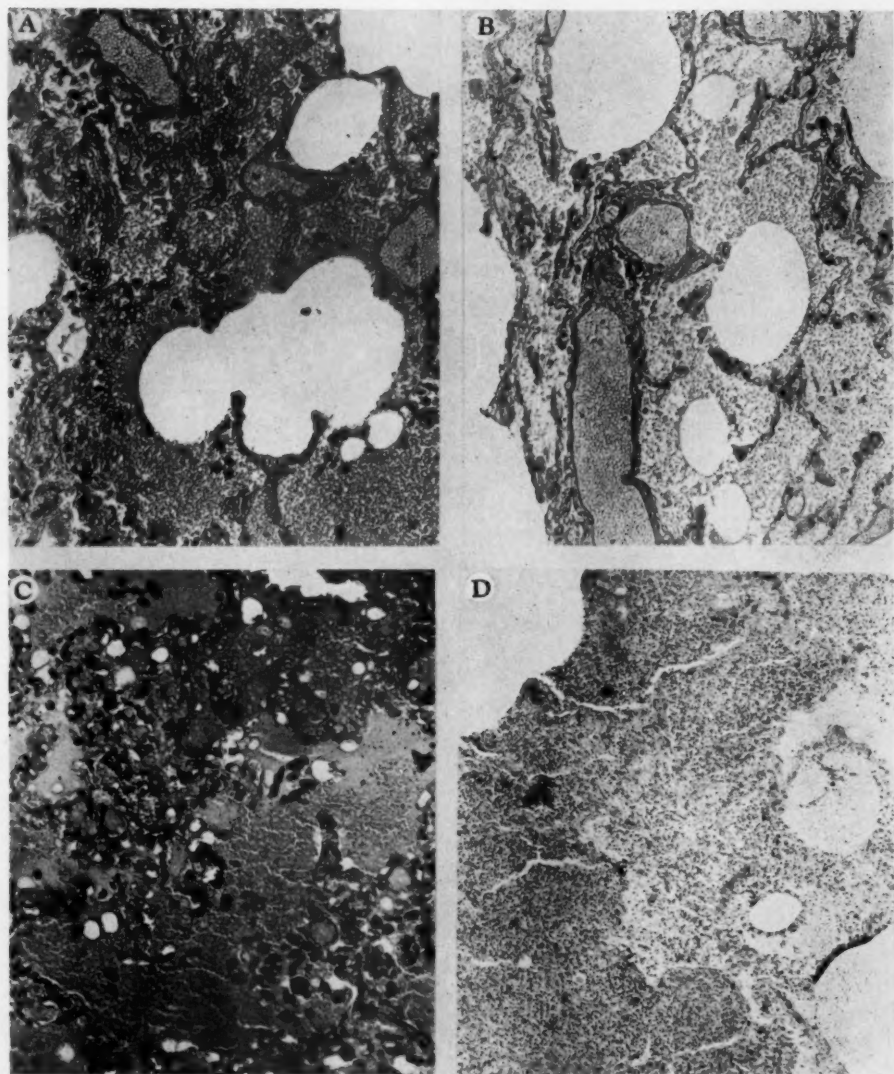


Fig. 3.—Photomicrographs of human lungs: formalin-fixed, paraffin-embedded, hematoxylin-cosin-stained; reduced to 73% of magnification $\times 115$.

A, strangulation, woman; contains stringy and granular edema fluid, medium congestion, and fine alveolar phagocytes.

B, paraldehyde intoxication, man; contains stringy and granular edema fluid, heavy congestion, and a very few alveolar phagocytes.

C, electrocution, man; contains diffuse smooth edema fluid, heavy diffuse hemorrhage, and many alveolar phagocytes.

D, drowned, man; contains sand throughout the alveoli, some stringy edema fluid, and alveolar walls are difficult to locate (same case as that in Fig. 1C).

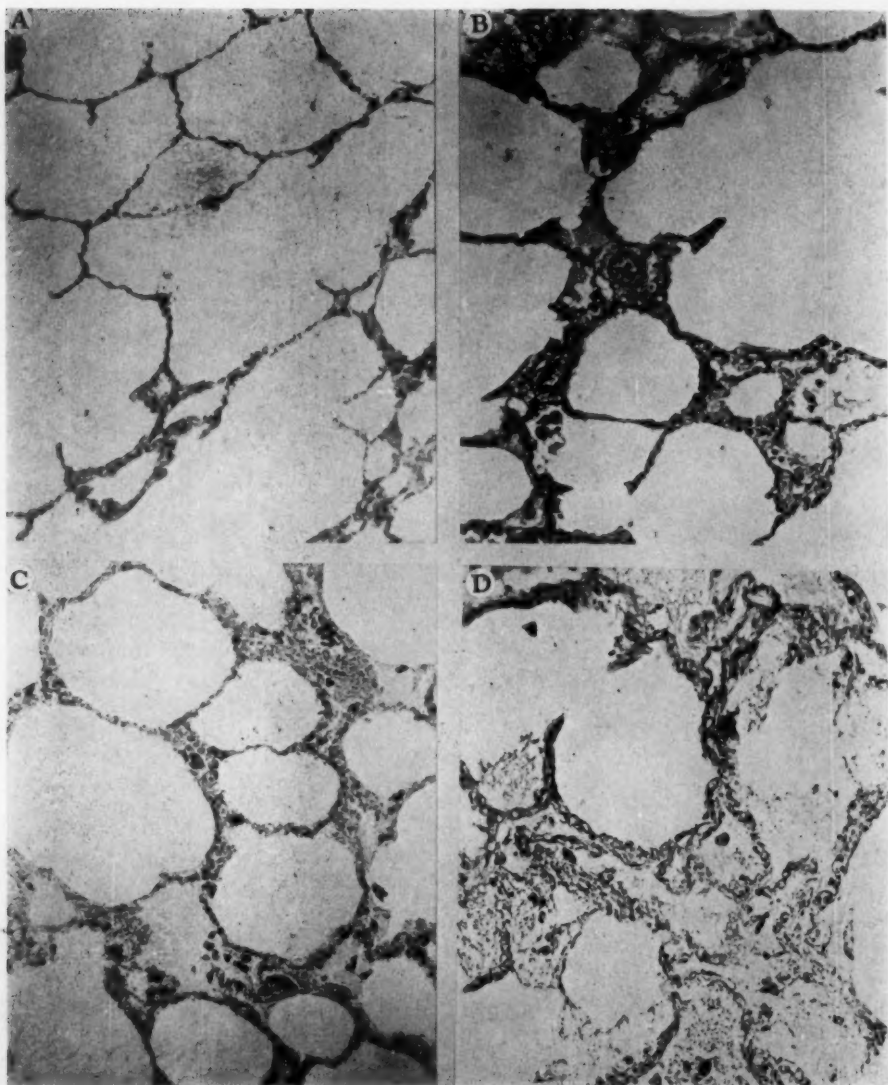


Fig. 4.—Photomicrographs of human lungs: formalin-fixed, paraffin-embedded, hematoxylin-cosin-stained; reduced to 73% of magnification $\times 115$.

A, exsanguination, male, normal.

B, drowned, man; contains light edema fluid and a few alveolar phagocytes.

C, brain injury, man; light granular edema and a few alveolar phagocytes.

D, drowned, woman; contains stringy edema and a few alveolar phagocytes.

head, the presence of sand was most noticeable in some sections of the lung and had been aspirated into the very terminations of the alveoli. Sometimes the edema and sand appeared together (Fig. 3D), and sometimes this alternated with areas where only edema appeared (Fig. 1C).

The most noticeable feature of the lung tissue sections from the drowned humans was pulmonary edema. The edema fluid was stringy or granular and was distributed in a patchy fashion. Alveolar phagocytes were present in greater quantities than expected. The lymphatics were not dilated, and there was an absence of hemorrhage and vascular congestion.

Cardiac Failure.—All 15 persons had had little or no previous treatment for heart disease. All had died suddenly, on the street or in their homes or at work. There was only one case with free alveolar hemorrhage (Fig. 2B). The history revealed that this person had been treated for cardiovascular disease. No further clarification could be obtained. The sections also contained medium edema, heavy congestion, and heavy alveolar phagocytes. In one case, with a history of hypertension, the cause of death was not definitely established. The lung sections were considered normal and contained no edema or congestion, and/or hemorrhage, but there were a few alveolar phagocytes. This case was included in this group because of the hypertension. The balance of the cases had sections that were uniform in appearance. In these the edema was light to medium, while the congestion was medium to heavy; there was no hemorrhage, but alveolar phagocytes ranged from light to heavy. Figure 2A is somewhat representative of these 13 cases.

In 14 cases tissues contained edema and congestion, and in all 15, alveolar phagocytes, while there was only 1 case with hemorrhage. The most noticeable feature in these lungs was the amount of congestion. This was observed in almost every microscopic field and involved all vessels. The edema, while not severe, had a granular appearance in most sections. The distribu-

tion was not as patchy as in the cases of drowning, and in many cases the edema fluid appeared smooth and completely filled the air spaces.

Brain Injury.—These 15 cases included skull fractures which resulted from falls, and craniocerebral damage of gunshot wounds or automobile accidents, and ruptured aneurysms of cerebral arteries. Most of these deaths occurred in a matter of minutes, and all of them within an hour or two of the loss of motor activity.

The skull fractures presented a near-uniform pattern of light to medium edema, medium congestion, medium hemorrhage, and light to medium alveolar phagocytes. The balance of the cases had lesions which ranged from light to heavy. One of these cases had no edema or hemorrhage; another had no alveolar phagocytes, and three others had no hemorrhage.

The outstanding features of the brain injury cases were the congestion and hemorrhage. All of the cases had sections containing congestion, and 11 had sections of hemorrhage. The congestion was similar to the cardiac cases but in most instances not as severe. The severe hemorrhage was the most striking feature of these cases. Fourteen cases had edema and alveolar phagocytes. The edema fluid was either stringy or granular, as in the drowning cases. The area shown in Figure 2C is somewhat representative of the group.

Strangulation.—All nine deaths were suicidal hangings, none of which resulted in a broken neck. Congestion was present in sections from all cases and was similar to cardiac and brain injury cases. One case with severe congestion is presented in Figure 2D. Eight had edema that was of a light degree in most instances. Hemorrhage was present and severe in one tissue section of one case. This was in the middle right lobe, and it was thought to be aspirated. Alveolar phagocytes were observed in seven persons.

These strangulation tissues showed little difference from those of cardiac deaths. Congestion was prominent in all cases but

not always as marked as in the cardiac victims. The edema was similar to that of drowning, with the same irregular distribution and stringy or granular appearance. There was an absence of hemorrhage in these cases.

Miscellaneous Injury.—The cases were described individually as nearly every case differs from the other, and one may refer to the Table for comparisons. The sections from the exsanguinated cases were normal. The lungs of the phosphorus ingestion case likewise were normal; however, there were some alveolar phagocytes. The lungs of the barbiturate case were also normal. The kind and amount of barbiturates taken in this incidence were not known; however, the dose produced cessation of breathing while the patient slept.

The lungs of the three cases just cited were included for comparison. The lungs of the meperidine (Demerol) suicide contained heavy congestion and phagocytes but light edema, whereas the pentobarbital suicide had lungs with medium edema, congestion and phagocytes.

Two carbon monoxide victims had tissues with medium edema and congestion. The phagocytes of one of these were light and the other medium. The third victim of carbon monoxide had inhaled flame, and the edema and congestion were severe. The alveolar phagocytes in this case were few.

Edema was light in the lungs of the paraldehyde, phenol, and mercury bichloride ingestion cases. Congestion was heavy in the lungs of the paraldehyde and phenol cases but absent in the lungs of the mercury bichloride cases. The phagocytes were heavy in the paraldehyde case but medium in the other two. The mercury bichloride lung sections had severe hemorrhage.

The two electrocution victims had lung tissues that were somewhat dissimilar. One had marked edema and hemorrhage, while the other had very little edema and no hemorrhage. Both had medium congestion and heavy phagocytes.

In the suffocation case the lung sections contained light edema, congestion, and phagocytes.

Considering the 16 miscellaneous cases as a group, there were 2 cases with hemorrhage, 12 cases with congestion, 13 with edema, and 14 with alveolar phagocytes. Three patients had lungs that were considered normal.

Summary of the Microscopic Observations.—Seventy cases of sudden death have been included in this study. Sixty-four of these had lung tissue sections that contained pulmonary edema to some degree. The edema fluid appeared stringy or granular in most instances. There were also 64 cases containing alveolar phagocytes, but they were not always the same cases as those with edema. Congestion was observed in lung sections from 53 cases. Some cases had edema and no congestion, and some had congestion and no edema. Congestion was absent in the usual cases of drowning. However, it was a very noticeable feature of the cardiac and strangulation lungs. Hemorrhage was observed in the lung sections of 16 persons. Eleven of these persons were in the brain injury group.

A case was considered normal if all sections contained no edema, congestion, or hemorrhage, and alveolar phagocytes were not severe. Four cases met these conditions: a case of barbiturate narcosis, a case of phosphorus ingestion, a case of exsanguination, and one case listed as cardiac failure, with no findings except hypertension.

Comment

Of the 70 cases in this study, 64 had lung tissue sections that contained edema to some degree. The edema varied from the barely detectable to the nearly complete inundation of all alveolar spaces. The appearance of the edema did not always conform to the classical pathological description, in which the fluid is considered to be a homogeneous, pink-staining, cell-free coagulum that completely fills all alveolar spaces. The edema observed in lung tissue sections

from persons with various diseases may fit this description. In some cases in this study the lung tissue contained an edema fluid with a smooth hyaline appearance that fits the classical description. However, the edema fluid in these sudden deaths appeared in most cases as stringy or granular. In some cases the edema fluid appeared stringy in some areas and granular in other areas. In still other cases the edema fluid appeared only stringy. Martin⁴ has described the granular appearance of pulmonary edema in drowning. A search of the literature did not reveal any description of pulmonary edema in which the appearance was described as stringy.

Whether the different appearances of the edema fluid is an indication of its composition is not entirely known. However, it seems reasonable to suggest that the stringy edema fluid may be due to fibrin threads, while the smooth appearance may indicate that the edema fluid may contain albumen, since egg albumen gives a smooth appearance on microscopic observation. Hemoglobin released from erythrocytes also may contribute to the smooth appearance. Granular edema may indicate the presence of globulin in the edema fluid, as Swann⁵ has demonstrated the presence of large amounts of globulin in the edema fluid of drowned dogs. Thus, the differences in the appearance could represent the presence of different blood constituents.

The results of microscopic observations on the lung tissue sections from the human cases indicated that pulmonary edema occurred in a variety of sudden deaths. These included drowning, sudden cardiac failures, such brain injuries as skull fractures, craniocerebral injury, and ruptured aneurysms of cerebral arteries, strangulation, carbon monoxide poisoning, natural gas asphyxia, Freon gas asphyxia, suffocation, barbiturate overdose, electrocution, and the ingestion of paraldehyde, phenol, and mercury bichloride.

Lung edema has been reported in many, but not all, of the types of cases listed above. Many reports have been made of its occurrence in drowning, and Brouardel⁶

was probably the first to report it from microscopic observation. There have been more articles concerning pulmonary edema in cardiovascular diseases than in any other condition, and Flint⁷ has listed most of these cardiovascular diseases. Edema has only recently been mentioned by Hess⁸ in connection with coronary arterial diseases. Weisman⁹ has shown that severe edema and congestion of the lungs develop in cases of intracranial hemorrhage. In most of the cardiac failure and brain injury cases reported in the literature, the death has been slow and the edema termed chronic. Whether edema developed when death was sudden from similar conditions was not known. The results of this study show that edema was present at autopsy in most of the cases of sudden deaths. Whether it could be considered entirely a postmortem phenomenon is open to question, since some of the autopsies were made within two or three hours.

Although edema has been shown by Masius¹⁰ to occur in cases in which substances have lodged in bronchi or bronchioles and death ensued, it has not been reported in cases of strangulation by hanging. This may be because the congestion may overshadow the edema, as illustrated in Figure 3D. No reports were found of edema in paraldehyde, phenol, or mercury bichloride poisoning. Reimann¹¹ mentioned a case of iodine poisoning in which edema was observed at autopsy. He also listed edema in death from barbitol compounds and asphyxiating gases, but did not specify which compounds or which gases. The two barbiturate cases suggest that an overdose may be necessary to produce edema in the lungs. The Freon, natural gas, and carbon monoxide cases had sections with edema. The presence of edema in persons inhaling phosphorus compounds has been shown by Carlisle¹² and others; however, the microscopic observations on the phosphorus ingestion revealed no edema, or even congestion, of the pulmonary vessels. Thus, the formation of pulmonary edema by phosphorus compounds may depend upon the contact of phosphorus with the alveolar capillary membrane.

Not only did edema occur in many cases, some of which have not been reported before, but certain observations on the pathologic physiology of the various cases of asphyxial death were unique. Alveolar phagocytes, or macrophages, or desquamated epithelial cells, as referred to by Lang¹³ and others, or heart failure cells, as they are usually referred to, were found in almost every sudden asphyxial death. The hemorrhage in the lungs of brain injury cases was noticeable and unexpected as well as unexplainable. Congestion was as noticeable in the strangulation cases as in the cardiac failures, but the appearance of the edema was somewhat different. The edema fluid of cardiac failures was smooth or granular, whereas in strangulation it was stringy or granular.

The stringy edema fluid as seen in most drowning cases was distributed in a patchy fashion. The granular edema fluid had less of a patchy distribution while the smooth edema fluid was continuous. As acute edema of the lungs is a rather explosive process the fluid enters the alveoli from the vessels, quickly fills them, and pours out through the atrium into the alveolar duct. If an adjacent primary lobule does not contain edema, the air in this lobule may be trapped by the fluid which covers the atrium. Pattle¹⁴ has demonstrated that edema fluid has a high surface tension, which explains its ability to trap the air. Thus, the air in some areas and fluid in others accounts for the patchy distribution. In the formation of the smooth or hyaline type, the filling must be slower and the air allowed to escape, and consequently the edema is evenly distributed throughout. If the air trapped in the lobule is forced through the fluid by breathing movements, it will appear as foam or froth in the trachea or bronchi.

In drowning, with the exception of the edema present, the sections could be considered normal, since congestion and hemorrhage were absent. The edema of drowning must involve an outpouring of fluid from the vessels which is quite different from cardiac failure, brain injury, or strangula-

tion, where the whole complex of hemodynamics within the pulmonary circuit is undergoing an irreversible change from the blood stream to the alveolar spaces. In drowning, the water must first move into the blood before the blood elements move into the alveolar spaces. The water may move out into the tissues by circulation of the blood, or it may move back into the alveolar spaces. Since congestion does not occur in drowning, the pulmonary edema formation may involve less vascular hemodynamic complications than in other cases of sudden death. Most investigators agree that, in drowning, water passes into the blood stream and dilutes the blood constituents. Moritz¹⁵ has summarized the blood changes that occur during drowning. He states that whatever blood constituent is measured, after fresh-water drowning, the right-heart blood is more concentrated than the left-heart blood. On the contrary, after sea-water drowning, the right-heart blood is found to be more dilute than the left-heart blood. Durlacher, Freimuth, and Swann¹⁶ reviewed the literature on disproportionate intracardiac hemodilution and measured plasma and whole blood specific gravity in 39 human drowning deaths. They found that, in all cases of drowning, irrespective of the salinity of the water, the specific gravity of the left-atrial plasma was less than that of the right-atrial plasma. In further studies, using 80 drowning and 80 other asphyxial deaths, Freimuth and Swann¹⁷ have shown that in drowning the left-atrial plasma specific gravity is less than the right-atrial plasma specific gravity. However, in the other asphyxial cases about half of them had lower specific gravity of the right-atrial plasma and half of them had lower values in the left-atrial plasma. These differences in asphyxial cases were slight as compared with the differences in specific gravity of drownings.

The observation in this study was made on lung tissues of subjects that had postmortem intervals of 2 to 20 hours. These appearances could be due to postmortem changes; however, if edema occurs in disease conditions

PULMONARY EDEMA IN SUDDEN ASPHYXIA

and is observed at autopsy in a relatively short period after sudden death in persons known to have no previous edema-producing conditions, then it seems logical it could develop during the period when the person is actually dying, i.e., the agonal period or that time between cessation of breathing and cessation of heart action. Since observations and measurements cannot ethically be made on humans at the time of death, it becomes necessary to resort to animal experiments in order to determine whether edema can be produced in sudden asphyxial deaths after cessation of breathing and before cessation of heart action.

Summary

A study was made of the lungs of 70 human victims of sudden asphyxia. Autopsy was performed as soon as possible after death. The cases included death by drowning, cardiac failure, brain injury, strangulation, carbon monoxide, Freon gas, natural gas, suffocation, barbiturates, electrocution, exsanguination, and the ingestion of paraldehyde, phosphorus, phenol, and mercury bichloride. Pulmonary edema was observed in 64 cases. It was not found in a case of strangulation, brain injury, barbiturate narcosis, phosphorus ingestion, and exsanguination, and in a case with no specific cause of death and only a medical history of hypertension.

The edema does not always appear as presented by the classical pathological description of a smooth, homogeneous pink-staining coagulum that completely fills all alveolar spaces. In these sudden asphyxial deaths the edema fluid appears mostly stringy or granular and was distributed in a patchy manner throughout the lungs. The amount of edema ranged from the barely detectable to the near-complete inundation of all alveoli.

Each type of death had distinguishing features. Congestion was prominent in strangulation, cardiac failures, and brain injury. Hemorrhage was a marked feature of brain injury cases. Edema was the out-

standing feature of drowning, carbon monoxide poisoning, natural gas asphyxia, and Freon inhalation. The edema in drowning was unaccompanied by vascular congestion or hemorrhage.

Pulmonary edema occurred in a variety of these sudden asphyxial deaths, even when autopsy was within two hours post mortem. These included drowning; sudden cardiac failures; such brain injuries as skull fractures, craniocerebral injury, and ruptured aneurysms of cerebral arteries; strangulation, carbon monoxide poisoning; natural gas asphyxia; Freon gas asphyxia; suffocation; barbiturate overdose; electrocution, and the ingestion of paraldehyde, phenol, and mercury bichloride.

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REFERENCES

1. Henneman, P. H.: Acute Pulmonary Edema, with Special Reference to Experimental Studies, *New England J. Med.* 235:590 (Oct. 17) 1946.
2. Durlacher, S. H.; Banfield, W. G., Jr., and Bergner, A. D.: Post-Mortem Pulmonary Edema, *Yale J. Biol. & Med.* 22:565, 1950.
3. Miller, W. S.: *The Lung*, Springfield, Ill., Charles C Thomas, Publisher, 1937.
4. Martin, E.: Les Lésions du foie dans la mort par submersion, *Ann. méd. lég.* 12:372, 1932.
5. Swann, H. G.: Studies in Resuscitation, U.S.A.F. Air Material Command Memorandum Report No. MCREXD69679J, March, 1949, p. 4.
6. Brouardel, P.: *La Pendaison, la strangulation, la suffocation et la submersion*, Paris, J.-B. Bailliere et Fils, 1897.
7. Flint, A.: *Physical Exploration and Diagnosis of Disease Affecting the Respiratory Organs*, Philadelphia, Blanchard & Lea, 1856.
8. Hess, W. R.: Die Regulierung des Blutkreislaufes, gleichzeitig ein Beitrag zur Physiologie der vegetativen Nervensystems, Leipzig, G. Thieme, 1930.
9. Weisman, S. J.: Edema and Congestion of Lungs Resulting from Intracranial Hemorrhage, *Surgery* 6:722, 1939.
10. Masius, V.: Pathogenie des oedemes pulmonaires aigus, 13th Congress International de Medecins, Paris, 1900, p. 179.

11. Reimann, H. A.: Diseases of the Lung, in *A Textbook of Medicine*, Ed. 5, edited by R. L. Cecil, Philadelphia, W. B. Saunders Company, 1940.
12. Carlisle, J. M.: Pulmonary Edema, *J.A.M.A.* 123:947, 1943.
13. Lang, F. J.: Über Gemebkulturen der Lunge, *Arch. exper. Zellforsch.* 1926, p. 2.
14. Pattle, R. E.: The Properties of the Foam of Acute Lung Oedema as Evidence of the Nature of the Alveolar Lining, *Porton Technical Paper*, No. 401, March, 1945.
15. Moritz, A. R.: Chemical Methods for the Determination of Death by Drowning, *Physiol. Rev.* 24:70, 1944.
16. Durlacher, S. H.; Freimuth, H. C., and Swann, H. E., Jr.: Blood Changes in Man Following Death Due to Drowning, with Comments on Tests for Drowning, *A.M.A. Arch. Path.* 56:454, 1953.
17. Freimuth, H. C., and Swann, H. E., Jr.: Plasma Specific Gravity Changes in Sudden Deaths: Observations with Special Reference to Drowning, *A.M.A. Arch. Path.* 59:214, 1955.

Reaction of Chorioallantoic Membrane of Chick Embryo to *Nocardia Intracellularis* Inoculation

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Nocardia intracellularis was isolated and its characteristics comprehensively studied by Cuttino and McCabe.¹ In man the disease produced by this organism is a pure form of granulomatous inflammation, characterized by invasion of the reticuloendothelial cells by the pathogen and proliferation of these cells. Massive intracellular parasitism may occur and an apparent equilibrium result between the organism and the cells of its host.

In the past, numerous pathogenic acid-fast organisms have been isolated by many workers and their pathogenic potentialities studied in comparison with those of human, bovine, and avian tubercle bacilli and *Nocardia* (Forbus et al.,² Richardson et al.,³ Timpe and Runyon,⁴ Pollak and Buhler⁵). For the purpose of this study, *N. intracellularis* and its chemical fractions were applied to the chorioallantoic membrane of chick embryos. This medium has been used extensively for culture of organisms since it was described by Borrell (1905) and Levaditi (1906).⁶ Only a short time is required to produce lesions or to cultivate an organism, and in this relatively young tissue it is very convenient to analyze the inflammatory process provoked.

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Materials and Methods

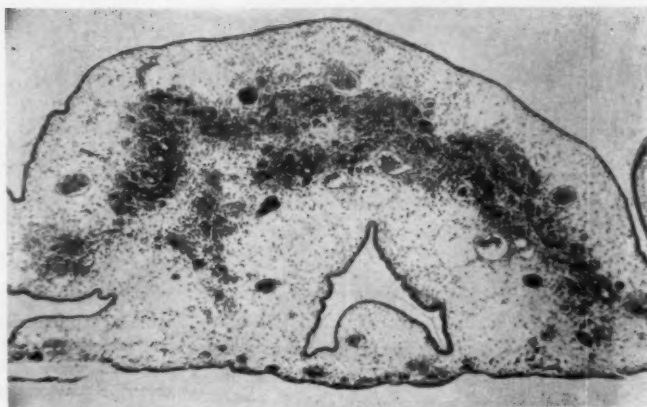
The technique of inoculation employed was essentially the same as that described by Goodpasture and Anderson⁷ and by Anderson.⁸ *N. intracellularis* organisms were placed on Petragani slants, and also in bottles containing about 200 cc. of modified Proskauer-Beck synthetic medium. Three weeks after inoculation, isotonic saline suspensions were made of the organisms cultivated on Petragani slants, so that 0.1 cc. contained 0.001 or 0.005 mg. of the organisms. In the same manner, suspensions of heat-killed organisms were made.

Three months after inoculation, waxes, acetone-soluble fat, and phosphatide were extracted from the organisms in the synthetic medium according to Anderson's method.⁸ The polysaccharide was extracted after the method of Palmer and Gerlough.⁹ The defatted organisms were obtained during the procedure of lipid extraction. Suspensions contained 0.001 or 0.005 mg. of defatted organisms or chemical fractions extracted from them. After sterilization each suspension was placed on the chorioallantoic membranes of 8- or 9-day-old embryonated eggs. The eggs of each group were studied during the immediately following 312 hours. From each egg a piece of membrane was spread on a slide and stained with neutral red and Janus green. Another piece of the membrane was fixed in Zenker's solution or 10% formalin. Serial paraffin sections were made and stained by a variety of techniques, as follows: hematoxylin-eosin, Ziehl-Neelsen, Gram, Masson (connective tissue), Wilder (silver impregnation), Unna-Pappenheim (plasma cell), and periodic acid-Schiff.

Observations

Reaction to Living, Heat-Killed, and Defatted Nocardia Intracellularis.—Immediately following inoculation the changes seen on the membrane surface were hyperemia, edema, and focal hemorrhage. Most likely these changes were caused partially by mechanical irritation due to opening of the shell. Within three hours after inoculation neutrophilic and eosinophilic leukocytes ap-

Fig. 1.—Photograph at low magnification of a typical reaction in the chorioallantoic membrane of the chick following inoculation with heat-killed *Nocardia intracellularis* organisms (0.01 mg.) 168 hours after inoculation. The inflammatory reaction is characteristically perivascular. The cells are chiefly mononuclear, although polymorphonuclear leukocytes are numerous. Here and there are clusters of mononuclear cells with beginning epithelioid alteration indicative of the early stages of true granuloma formation.



peared on the surface of the ectodermal epithelium and between the covering cells. In places the epithelium was lost and leukocytes appeared in the subectodermal layer. The nuclei and cytoplasm of the covering cells were swollen, and small cytoplasmic vacuoles were present. Mitotic figures were present in small number. The subectodermal capillaries appeared somewhat congested, and they contained a few eosinophilic leukocytes and mononuclear cells. The latter were characterized by basophilic cytoplasm and large round nuclei with one or two large nucleoli.

After 24 hours a proliferation of spindle or asteroid cells with delicate cytoplasmic projections took place, and these cells showed an irregular or palisade arrangement. These foci were usually surrounded by proliferating capillaries communicating with branches of subectodermal capillaries. Frequently the reaction spread through the mesodermal tissue as far as the endoderm. At the surface of the lesions, the ectodermal hyperplasia resulted in elevation and focal stratification of the epithelium. There was occasional hemorrhagic necrosis, with ulceration involving even the superficial layer of the mesodermal tissue. Proliferation of the ectodermal components extending toward the subectodermal layer and resulting in isolated "epithelial pearl formation," as described by Moore,¹⁰ was

sometimes noted. The origin of the associated young granulation tissue seemed to be especially the undifferentiated connective tissue cells attached to the basal membrane of the ectodermal layer and the capillaries beneath the ectodermal tissue. Delicate argyrophilic fibers were identified in the granulation tissue with Wilder's silver stain. There were many mitotic figures. Some eosinophilic leukocytes and a few mononuclear cells were interspersed among the other cells. The number of these cells in the blood vessels was increased. The cytoplasm of the young connective tissue cells was swollen and vacuolated, and it contained Schiff-positive materials. Some erythrophagocytosis by macrophages was noted.

During the following 24 to 48 hours mononuclear cells increased both in and around the capillaries. These cells contained many small Janus green granules and a few vacuoles. The proliferating ectodermal epithelium exhibited vacuolar and hydropic cytoplasmic changes, sometimes taking on the appearance of a little vesicle.

In 48 hours small nodules, composed of accumulations of eosinophilic or basophilic granule-laden mononuclear cells and large leukocytes, became conspicuous around the vessels. Mitotic figures were numerous in the mononuclear cells. All types of inflammatory cells contained phagocytosed or-

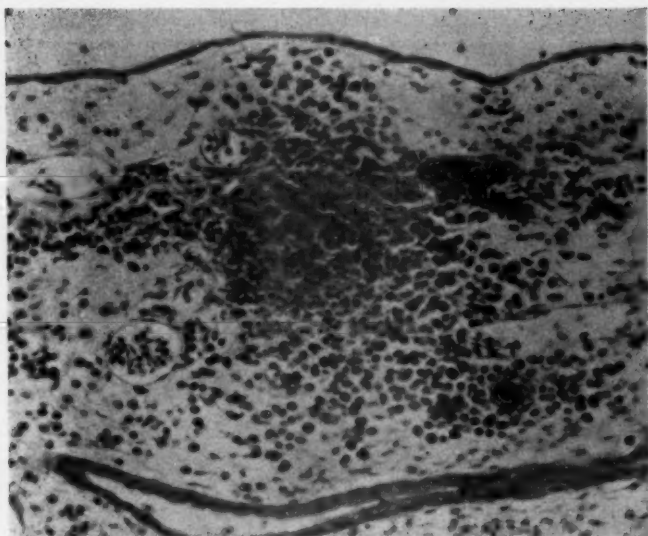


Fig. 2.—Photograph at medium magnification of a typical early reaction of the chorioallantoic membrane of the chick following inoculation with living *Nocardia intracellularis*. The reacting cells are almost exclusively mononuclear. At the center is a focus of necrotic cells which had undergone early epithelioid alteration.

ganisms and Schiff-positive material. A few multinucleated giant cells were present in the mononuclear-cell aggregates, but these were quite different from the usual Langerhans giant cells.

In the 72-hour preparation the round-cell accumulation around the capillaries had increased greatly. These cells maintained their oval or round shape and were noteworthy for their homogeneous basophilic cytoplasm and round, hyperchromatic nucleus with encircling halo. Metachromasia of the cytoplasm was an outstanding feature. Many of these cells gave a positive reaction to the Unna-Pappenheim stain, identifying them as plasma cells. Most of the cells in the aggregates resembled blast forms in mitosis.

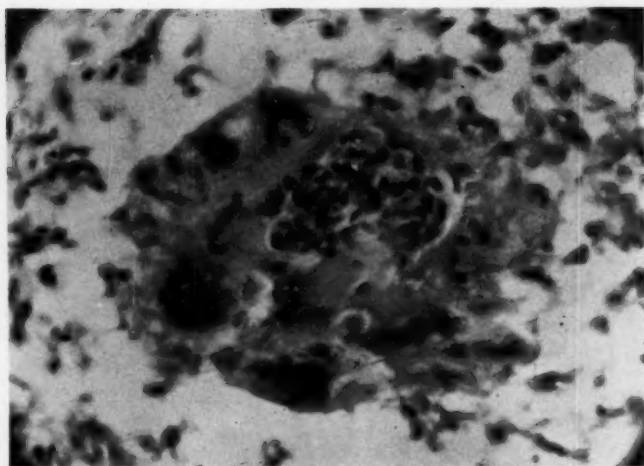
In general, the inflammatory reaction spread throughout the mesodermal tissue in 96 to 120 hours, and the degree of extension was directly related and proportioned to the concentration of organisms.

The invaginated ectodermal tissue provided a favorable site for the growing organisms, and sometimes the accumulation of leukocytes in the lumen of the invagination became so massive as to form an abscess-like lesion. Although liquefactive

necrosis regularly occurred in the epithelium and spread into the surrounding mesodermal tissue, in some places the basal membrane became interrupted and obscure, and proliferation of the ectodermal covering cells occurred and extended into the surrounding tissue. When these cells became isolated in small masses, as was their tendency, they assumed the appearance of polymorphonuclear giant cells. Adjacent to the ectodermal layer, a striking proliferation of spindle-shaped cells took place; some of these cells were distinctly epithelioid in their over-all character. An infiltration of eosinophilic leukocytes accompanied these reactions, the periphery of which was always characterized by a zone of mononuclear cells, many of which were undergoing disintegration. The reaction to the heat-killed and the defatted organisms was regularly less intense than that which followed inoculation of living organisms, and the lesion usually was epithelioid in appearance.

As the preparation aged, the ectodermal surface dried and thickened, eventually taking on the appearance of a layer of keratin. The underlying mesoderm became thick and fibrous. Although organisms were al-

Fig. 3.—Photograph of a genuine granuloma which resulted from inoculation of the chorioallantoic membrane of the chick with 0.01 mg. of *Nocardia intracellularis*. Duration of the infection, 232 hours.



ways demonstrable in the preparations, the reaction of the chorioallantoic membrane varied greatly, being minimal and confined to the surface in some of the embryos.

Extension of the organisms into the mesoderm seemed to take place by direct invasion from the inoculated surface of the ectoderm, by migration, or by transportation of organisms within macrophages. The last mechanism was especially conspicuous following inoculation of the heat-killed and defatted organism. The transporting cells seemed highly resistant to their cargo of organisms, which usually packed their cytoplasm, forming a rosette-like arrangement quite like that observed in the lymph nodes of the human case from which *N. intracellularis* was originally isolated. These organism-laden macrophages almost always contained a moderate amount of Schiff-positive material.

Reaction to Chemical Fractions of Nocardia Intracellularis.—A. Purified Wax: Focal hemorrhage and proliferation of the connective tissue cells around the capillaries appeared beneath the ectodermal tissue nine hours after injection. The young connective tissue cells gradually increased in number, and some of these contained more refractile vacuoles than are ordinarily noted in this type of cell. Eighteen hours after injection mononuclear wandering cells appeared.

Occasionally erythrophagocytosis was observed. Forty-eight hours after inoculation phagocytosis became active, especially around the vessels. Polymorphonuclear leukocytic emigration was relatively slight.

B. Acetone-Soluble Fat: Most impressive tissue injury was produced by this complex material 12 hours after its inoculation. Young connective tissue cells appeared and rapidly became swollen and vacuolated. This reaction was accompanied by the accumulation of wandering cells and these, too, quickly underwent necrotic alteration. In time, the supporting connective tissue in the mesoderm became edematous and lost its normal staining properties. Ninety-six hours after injection liquefaction of the connective tissue occurred, accompanied by coagulative changes in the ground substance, giving to the tissue a fibrinoid appearance. Polymorphonuclear leukocytic emigration was not remarkable.

C. Phosphatide: Twenty-four hours after inoculation, the cytoplasm of the proliferating young connective tissue cells became vacuolated and actively phagocytic. An impressive emigration of basophilic, nongranular mononuclear cells was present. These were accompanied by a few eosinophilic granule-laden mononuclear cells, and an occasional mono- or multinucleated giant cell. This reaction was most prominent

about the blood vessels. None of these cells exhibited the characteristic coarse, vacuolated cytoplasm of the "phosphatide cell," as described by Sabin.¹¹⁻¹³ There was no formation of epithelioid-cell tubercles, such as described by Sabin and by Roulet and Bloch^{14,15} in their studies of the reaction to the phosphatide fraction of the tubercle bacillus.

D. Polysaccharide: Three hours after inoculation a massive extravasation of eosinophilic leukocytes was observed. As time passed, these leukocytes increased in number and widely infiltrated the tissue. Twelve hours after inoculation plasma cells appeared on the scene. Basophilic, non-granular large mononuclear cells were observed in the capillaries and around the vessels situated beneath the ectoderm. The connective tissue cells proliferated, and occasionally assumed a rounded form, thus resembling macrophages. Seventy-two hours after inoculation, mononuclear cells collected in the lymph spaces and formed large aggregates about the blood vessels.

Comment

In this study the ultimate objective was an extension of our understanding of the granulomatous form of inflammation; in the last analysis, a further development of our concepts of the reaction potential of the cells of the reticuloendothelial system to animate and inanimate provoking agents.

N. intracellularis was chosen for study as a provocative agent for two reasons. First, it has an extraordinary capacity for stimulating the response of the reticuloendothelial cell, as demonstrated so impressively in the infection in man from which it was originally isolated. Second, it has a very limited capacity in its living form to provoke the reticuloendothelial cells which it attracts in human tissues to utilize those other reaction potentials which, when active, result characteristically in classic epithelioid and tubercloid granulomas, typified by what is familiar to all in tuberculosis.

In selecting the chick embryo as host we had three things in mind.

In the first place, we knew from the experiments of Cuttino and McCabe carried out in their original isolation and identification of *N. intracellularis* that this organism would grow satisfactorily in the chick chorioallantoic membrane and that the resulting infection would proceed slowly and last long enough to permit careful, almost hour-to-hour observation of the responses of the inflammatory cells.

In the second place, the use of this embryonic medium offered the possibility of studying the reactions of reticuloendothelial cells which are unmistakably embryonic and of comparing these reactions with those of human mature reticuloendothelial cells which we conveniently describe as "embryonic" because of their extraordinary reaction potential. Since cellular activity, regardless of its character, must be analyzed in terms of the chemical mechanisms which actually constitute the cell as a biological unit, it is not unfair to assume that truly embryonic reticuloendothelial cells might not necessarily possess certain ones of those chemical integrations which characterize the mature reticuloendothelial cell and account for its multiple reaction potentials, as exhibited in the fully developed granulomatous inflammatory reaction. This possibility is indeed in harmony with what we know about certain undifferentiated or partly undifferentiated cells of the mature person. For example, the cells of immature blood-forming tissue surely do not possess all of the enzymes or enzyme systems normally present in the cells of these tissues, a deficiency indicated by the fact that islands of immature blood formation resist autolysis in the dead body to a remarkable degree, far beyond that of the mature cells of the same body.

Thirdly, the use of the chick embryo offered the possibility at least of gaining a little more definite information about the origin and functions of the reticuloendothelial cell which appears on the scene with such speed and in such great numbers when appropriate provocative agents are introduced into the tissues. If we are to continue

to follow the line that the monocyte, the histiocyte, and the large mononuclear wandering cell of granulomatous inflammatory reactions are indeed the same cell, the macrophage, which we refer to specifically as the reticuloendothelial cell—as Aschoff and Kiyoo did—then the differences in morphology, size, staining reaction, movements, etc., of these cells should no longer give us concern, for the simple reason that these differences may merely indicate variations in one and the same cell associated with changing functional status. Nevertheless, in this study an attempt was made to differentiate the mononuclear cells in the area of infection of the embryonic tissue by appropriate staining methods. This, however, proved so difficult and eventually inconclusive that it seemed best in the end to give up the attempt and refer to all the responding forms simply as large mononuclear wandering cells.

We are not at all confident that we have established anything of significance in pointing out the cytologic responses to the several chemical fractions which were obtained when *N. intracellularis* was extracted. Our results are essentially comparable to those of Sabin et al. obtained in their fractionation studies of the tubercle bacillus and those of Baker and Bryan in their fractionation studies of *Blastomyces dermatitidis*. All these studies merely emphasize the fact that the provocation of cells to activity of whatever kind is essentially a chemical effect, and that such chemical effects may be highly specific. Were it not for this high degree of specificity, all granulomatous inflammatory reactions would be exactly alike morphologically and cytologically. But we know that this is not so. For example, in tularemia, a typical granulomatous disease, classic tubercles are never seen. The lesion is typically epithelioid. Again, in tuberculosis any or every phase of the morphologic reaction potential of the reticuloendothelial cell may be seen. All of this would appear to indicate that the matter of specificity of chemical stimulation of the reaction potential of cells of the reticuloendothelial system is far more com-

plex than would be indicated merely by saying that the waxy, acid-fast coat of the tubercle bacillus stimulates the reticuloendothelial cell to produce classic tubercles. Experience with the natural disease processes would, rather, suggest that a whole range of chemical provocateurs, acting at proper times and in definite sequence, is required to produce the classic tubercle. Our studies with the fractions of *N. intracellularis* have not helped us very much toward an understanding of this complex relationship.

Summary

Living, heat-killed, and defatted *Nocardia intracellularis* and some of its chemical fractions were deposited on the chorioallantoic membrane of the chick embryo.

A constant feature of the inflammation produced by this organism was a marked proliferation and phagocytic activity of mononuclear cells and exudation of polymorphonuclear leukocytes.

The acute focal lesions appeared to arise from a proliferation of mononuclear cells following an accumulation of leukocytes.

Spread of the inflammatory process into the mesoderm was often preceded by proliferation of the ectodermal cells.

Following the ectodermal tissue damage, the principal reaction in the mesoderm was fibroblastic proliferation.

The formation of giant cells of macrophage type was provoked by the injection of living organisms.

Injection of living and heat-killed defatted organisms resulted in an epithelioid reaction without the formation of typical tubercles.

Heat-killed and defatted organisms produced somewhat less tissue damage than living organisms.

The acetone-soluble fat fraction caused necrosis and hemorrhage.

The polysaccharide fraction provoked emigration of polymorphonuclear leukocytes, a few mononuclear wandering cells, and a minimal proliferation of connective-tissue cells.

The phosphatide fraction provoked some proliferation of connective tissue cells and the emigration of monocytes.

The wax fraction stimulated connective tissue cells to proliferate and stimulated a minimal response of mononuclear cells.

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REFERENCES

1. Cuttino, J. T., and McCabe, A. M.: Pure Granulomatous Nocardiosis: A New Fungus Disease Distinguished by Intracellular Parasitism; a Description of a Hitherto Undescribed Organism, *Nocardia Intracellularis*, N. Sp., Including a Study of the Biologic and Pathogenic Properties of This Species, *Am. J. Path.* 25:1-47, 1949.
2. Forbus, W. D.; Cuttino, J. T.; Smith, A. G.; Margolis, A. M., and Reid, D. W.: A Comparative Study of 4 Groups of Acid-Fast Organisms, with Special Reference to Pathogenicity, *A.M.A. Arch. Path.* 66:1-9, 1958.
3. Richardson, F. M.; Clancy, C. F., and Crane, A. R.: Pathogenicity of Chromogenic Acid Fast Bacteria, *Bull. Ayer Clin. Lab.* 4:31-42, 1954.
4. Timpe, A., and Runyon, E. H.: The Relationship of "Atypical" Acid-Fast Bacteria to Human Disease, *J. Lab. & Clin. Med.* 44:202-209, 1954.
5. Pollak, A., and Buhler, V. B.: The Cultural Characteristics and Animal Pathogenicity of an Atypical Acid-Fast Organism Which Causes Human Disease, *Am. Rev. Tuberc.* 71:74-87, 1955.

6. Levaditi, C.: La Spirillose des embryons de poulet dans ses rapports avec la treponemose, héréditaire de l'homme, *Ann. Inst. Pasteur* 20: 924-938, 1906.
7. Goodpasture, E. W., and Anderson, K.: The Problem of Infection as Presented by Bacterial Invasion of the Chorio-Allantoic Membrane of Chick Embryos, *Am. J. Path.* 13:149-174, 1937.
8. Anderson, R. J.: The Chemistry of the Lipoids of Tubercle Bacilli, *Physiol. Rev.* 12:166-189, 1932.
9. Palmer, J. W., and Gerlough, T. D.: A Simple Method for Preparing Antigenic Substances from the Typhoid Bacillus, *Science* 92:155-156, 1940.
10. Moore, M.: The Chorio-Allantoic Membrane of the Developing Chick as a Medium for the Cultivation and Histopathologic Study of Pathogenic Fungi, *Am. J. Path.* 17:103-120, 1941.
11. Sabin, F. R.: Cellular Reactions to Tuberculo-Proteins Compared with the Reactions to Tuberculo-Lipoids, *J. Exper. Med.* 68:837-852, 1938.
12. Sabin, F. R.: Cellular Reactions to Fractions from Tubercle Bacilli, *Am. Rev. Tuberc.* 44:415-423, 1941.
13. Sabin, F. R.: Cellular Reactions to Defatted Tubercle Bacilli and Their Products, *J. Exper. Med.* 68:853-865, 1938.
14. Roulet, F., and Bloch, K.: Studien zur Histogenese des tuberkulösen Granuloms, *Arch. path. Anat.* 294:262-277, 1934.
15. Roulet, F., and Bloch, K.: Beiträge zur Spezifität der Entzündung mit besonderer Berücksichtigung des tuberkulösen Granuloms, *Arch. path. Anat.* 298:311-326, 1936.

Gross Estimation of Atherosclerosis in Aorta, Coronary, and Cerebral Arteries

A Comparative Study in Los Angeles and South India

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Studies conducted among various populations of the world, including the Chinese,¹ Japanese,² Okinawans,³ and Bantus of South Africa,⁴ have indicated that arteriosclerosis is less severe than in United States. The comparisons have usually been based on such indirect evidence as the crude death rates from cardiovascular disease obtained from vital statistics or the frequency of clinical complications of arteriosclerosis, such as coronary occlusion or cerebral vascular accidents. The many pitfalls inherent in any indirect system of assay make direct examination and quantitation of the lesions desirable. When direct methods have been employed, the degree of severity of atherosclerosis has usually been estimated by means of a grading system from 1 to 3 or 4,^{2,4} or the authors have merely stated their impressions gained from autopsies performed both in the United States and abroad.^{1,3} While such studies have served their purpose, the need for a more quantitative and universally applicable system is apparent.

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From the College of Medical Evangelists and the Los Angeles County Hospital (Dr. Hirst); the Harvard School of Public Health and the Veterans Administration Hospital, West Roxbury 32, Mass. (Dr. Gore), and the Christian Medical College, Vellore, South India (Drs. Hadley and Gault).

Gore and Tejada⁵ devised a method of assay based on gross inspection that has the advantages of being simple enough to be performed during a routine autopsy, greater precision than the usually employed systems, applicability to various arteries of the body, and results that are reproducible by different investigators.

Methods and Materials

The material consists of 773 unselected autopsies, 514 of which were performed at the Los Angeles County Hospital and 259 at the Christian Medical College in Vellore, South India. Included in the Los Angeles group were 398 Caucasians, 78 Negroes, 29 Mexicans, and 9 Orientals. The group is considered representative of the indigent population of a cosmopolitan American city. The cases from Vellore, South India, included 248 South Indians, 6 North Indians, and 5 Mongolians. After exclusion of four Europeans, the remainder were considered as a single group, although it must be admitted that more than one ethnic group is represented. Although detailed information is not available regarding the living habits of the South Indians, the diet is generally low in calories and deficient in protein and fat, both animal and vegetable. Rice is the staple item of diet, and the food is usually highly seasoned.

Both the Los Angeles and the India group are composed almost entirely of hospital patients who died of natural causes. The distribution of cases by age and sex is shown in Table 1. The patients in India were generally younger than those in Los Angeles, the largest number in the former area being in the 30- to 39-year age group, and in the latter area, in the 70- to 79-year age group. Observations of the extent and severity of atherosclerosis were based on inspection of the arteries studied, which included the aorta and coronary and cerebral vessels in Los Angeles, and the aorta and coronary arteries in India. For consistency, a single observer recorded observations in each geo-

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TABLE 1.—Distribution of Cases

Age	Los Angeles 514		South India 229	
	Male 285	Female 229	Male 182	Female 77
0-9	1	5	--	--
10-19	3	7	20	13
20-29	2	5	38	20
30-39	7	20	41	22
40-49	24	23	37	13
50-59	52	37	32	6
60-69	72	31	11	2
70-79	84	60	3	1
80-89	38	37	--	--
90-99	2	4	--	--

graphic area. In a trial comparison, these two observers performed estimates on 50 aortas of random ages and compared results. The difference in the mean of the estimated per cents of surface

involved was only 6% (i.e., 51% vs. 57%). Since the estimates by the observer from India were the higher of the two, the differences found in the severity of atherosclerosis in the two areas are probably actually greater than the data would indicate.

Tejada and Gore⁹ suggested that the estimates for extent and the relative proportion of various types of lesions be used to calculate an atherosclerotic index. The extent of involvement was indicated by a letter rather than the actual per cent, O indicating 0-5%; A, 6%-15%; B, 16%-33%; C, 34%-50%, and D, 51%-100%. A constant value was assigned each category. Since the majority of cases at the Los Angeles County Hospital fell in Category D, in which 51% or more of the surface was involved, the use of the numerical per cent obtained by estimation was considered to be more accurate and has been used in all cases in this study. According to Gore and Tejada, each of the four types of lesions is assigned a biologic

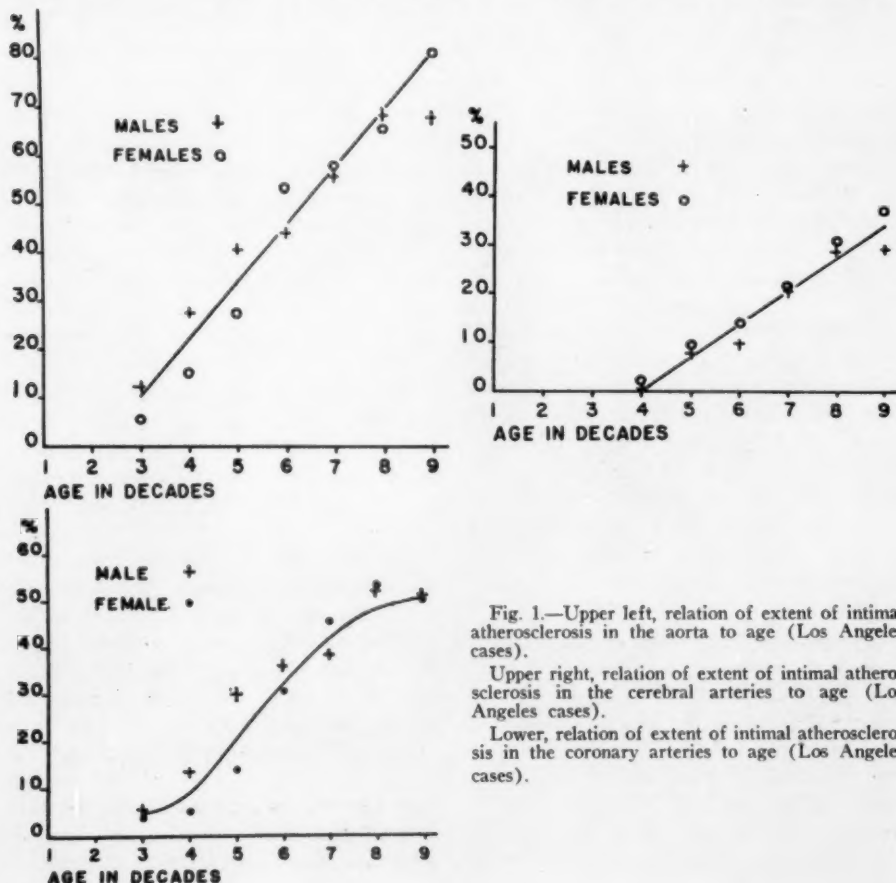


Fig. 1.—Upper left, relation of extent of intimal atherosclerosis in the aorta to age (Los Angeles cases).

Upper right, relation of extent of intimal atherosclerosis in the cerebral arteries to age (Los Angeles cases).

Lower, relation of extent of intimal atherosclerosis in the coronary arteries to age (Los Angeles cases).

weighting as follows: one-tenth (0.1) for lipid streaks; unity (1) for lipid or fibrous plaques, and times ten ($\times 10$) for ulcerative or calcific lesions, the last two being considered equally late lesions. Using the modification suggested above, the atherosclerotic index (A.I.) in this study was derived as follows:

$$\text{A. I.} = \% \text{ of intimal surface involved } \times [\text{lipid streaks} \times 0.1 + \text{plaques} \times 1 + \text{ulcerative lesions} \times 10 + \text{calcific lesions} \times 10]$$

The index so obtained is usually slightly lower than that obtained using the original procedure.

Results

Extent of Intimal Involvement by Atheroma.—The effect of age on the mean per cent of intimal surface involved by atherosclerosis in Los Angeles is shown in Figure 1, which includes (A) aorta, (B) cerebral arteries, and (C) coronary arteries. On each graph, the line has been fitted by inspection. The increase in intimal involvement of the aorta and the cerebral arteries with increasing age is essentially linear within the age groups studied. The coronary arteries show an S-shaped distribution curve, and it seems likely that a similar curve would have occurred in the aorta and cerebral arteries had there been a larger number of cases in the first 2 and the 10th decades of life. The increase in extent of intimal atheroma with age is most rapid in the aorta, less so in the coronary arteries, and least in the cerebral arteries. Definite sex differences are apparent in both the aorta and the coronary arteries, the extent of intimal involvement being less in the female below the age of 60 (third, fourth, and fifth decades) but subsequently rising to equal or exceed involvement in the male in both vessels. No sex difference is noted in the extent of cerebral artery atheroma.

Comparison of the mean extent of atheroma in the aorta in Los Angeles and India with the sexes combined (Fig. 2) revealed little difference in the two groups during the second and third decades. Beginning in the fourth decade, the extent of involvement was greater in Los Angeles and remained so thereafter. As in Los Angeles, the extent of intimal surface involved in

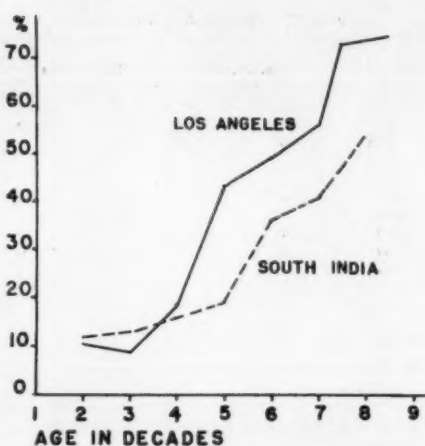


Fig. 2.—Comparison of the mean extent of involvement of the aorta by atherosclerosis in Los Angeles and South India.

India was greater in the aorta than in the coronary arteries, and the females had a lower mean per cent of intimal surface involvement of the aorta than the males.

The mean extent of intimal atheroma of the coronary arteries in males in Los Angeles was higher than in males in India in the third through the sixth decades, the decades with sufficient numbers for comparison. There were too few estimates of extent of coronary artery involvement in females in India for comparison.

Comparison of the Absolute Per Cent of Various Lesions.—In order to make a quantitative comparison of the various types of atherosclerotic lesions, the relative proportion of any lesion must be multiplied by the total per cent of intimal surface involved to obtain an absolute per cent of involvement. For example, if one-tenth of the lesions are calcific and 50% of the intimal surface is involved by lesions, then the absolute per cent of calcific lesions is $\frac{1}{10} \times 50$, or 5%. A graph showing the absolute per cent of each of the four types of lesions in the aorta in Los Angeles is shown in Figure 3. Lipid streaks reached their peak incidence in the second and third decades and declined thereafter. Plaques were first noted in the second and third

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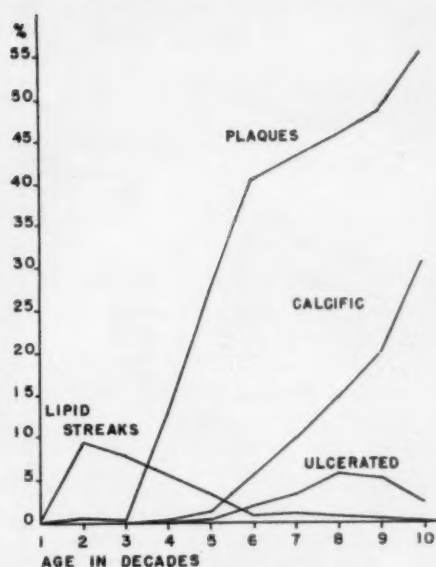


Fig. 3.—Relation of the absolute per cent of atherosclerotic lesions in the aorta to age in Los Angeles cases.

decades, and rose precipitously in the fourth decade. Calcific lesions appeared in the fourth decade and increased with a steep

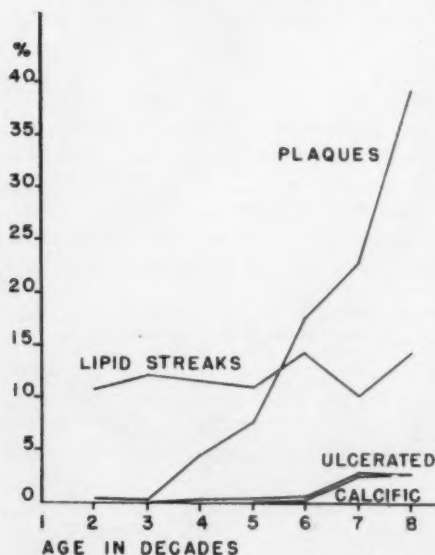


Fig. 4.—Relation of the absolute per cent of atherosclerotic lesions to age in South India cases.

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gradient thereafter. Ulcerative lesions, first observed in the fifth decade, rose gradually, reaching a low plateau in the eighth and ninth decades.

A similar graph of the India material is shown in Figure 4. Lipid streaks developed to about the same extent during the first three decades as in Los Angeles but, unlike the American group, did not decrease in subsequent decades. In India plaques occurred at a low level in the second and third decades, rising sharply in the fourth decade and onward, although not so precipitously as in Los Angeles. Ulcerative and calcific lesions first appeared in the fourth and fifth decades, respectively, and reached a low plateau in subsequent dec-

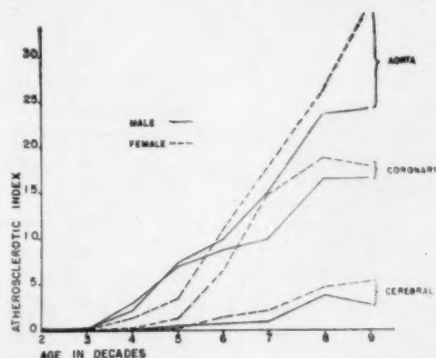


Fig. 5.—Comparison of the mean atherosclerotic index in the aorta and coronary and cerebral arteries in Los Angeles cases.

ades. The persistently low level of calcification of the aorta encountered in India in the sixth, seventh, and eighth decades contrasts with the dramatic increase encountered in the same decades in Los Angeles.

Comparison of the Atherosclerotic Index

Age and Sex.—A graph comparing the mean atherosclerotic index by age and sex in the Los Angeles area is shown in Figure 5. From the sixth decade and beyond, the index was highest in the aorta, lower in the coronary arteries, and lowest in the cerebral arteries. The female tended to have

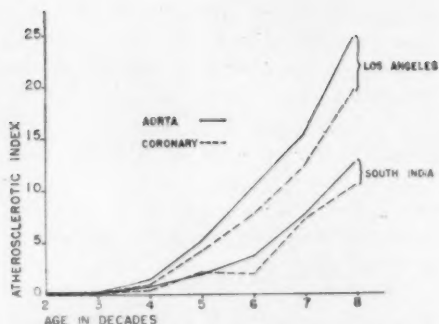


Fig. 6.—Comparison of the atherosclerotic index of the aorta and coronary arteries in Los Angeles and India (sexes combined).

a lower index in both the aorta and the coronary arteries during the first five decades, with a statistically significant ($t_{47}=3.64$) lower index in the coronary arteries of the female in the fifth decade. A sharp rise in the mean index of females occurred in the sixth decade, exceeding the index in the males in the subsequent three decades (60-89 years) in the three vessels studied.

Comparison of the atherosclerotic index in the aorta and coronary arteries in the two geographic areas with the sexes combined is shown in Figure 6. Beginning in the fourth decade, the atherosclerotic index was lower in both the aorta and the coronary arteries of the India group, the disparity increasing with age. Separating the sexes in the India group revealed a lower index in the aorta in the female in the third through the sixth decades. There were insufficient cases for comparison by sex above this age.

Effect of Hypertension.—Separation of the hypertensive from the normotensive cases in the Los Angeles group (Fig. 7) revealed that the higher index encountered in the aorta in the female during the later decades was not due to sex alone, since the normotensive female had essentially the same mean index as the normotensive male. Hypertension increased the atherosclerotic index in both sexes in the sixth, seventh, and eighth decades, the index of the hypertensive female being higher than that of

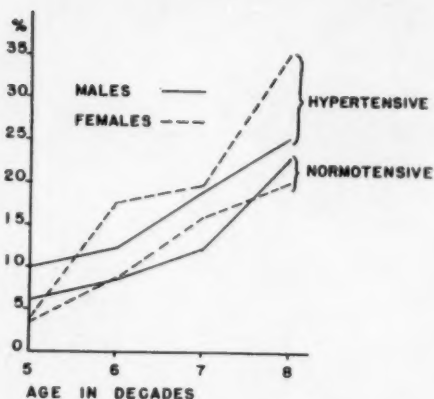


Fig. 7.—Effect of hypertension on the atherosclerotic index of the aorta in Los Angeles cases.

the hypertensive male. A higher index was found in both the coronary and the cerebral arteries of hypertensive patients during the same decades with the sexes combined.

Effect of Nutrition.—The relation of the state of nutrition as determined at autopsy to the atherosclerotic index of the aorta in the Los Angeles group is shown in Table 2, in which the sex groups are combined. A slight, but consistently lower, index is observed in the thin patients as compared with those of average weight; however, no consistent increase was noted in the overweight. Admittedly, the clinical records were frequently inadequate to indicate how many previously obese patients had become thin during their terminal illnesses. On the basis of autopsy, 46% were considered thin or malnourished, 33% as average, and 21% as obese. Observations regarding state of nutrition in India were

TABLE 2.—Effect of Nutrition on the Mean Atherosclerotic Index (Los Angeles Cases)

Age	Thin (46%)		Average (33%)		Overweight (21%)	
	Index	No. of Cases	Index	No. of Cases	Index	No. of Cases
40-49	4.5	10	5.66	30	4.07	6
50-59	11.52	36	12.9	38	6.67	18
60-69	12.70	41	16.40	41	19.37	21
70-79	23.66	77	25.90	28	24.61	39
80-89	25.91	41	33.1	21	35.87	13

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TABLE 3.—Effect of Race on the Mean Atherosclerotic Index in the Aorta (Los Angeles Cases)

		40-49		50-59		60-69		70-79	
		Index	No. of Cases	Index	No. of Cases	Index	No. of Cases	Index	No. of Cases
Aorta	White	7.17	25	11.3	53	17.6	80	24.4	60
	Negro	3.3	17	11.3	20	8.2	13	20.9	6
Coronary arteries	White	6.2	25	9.2	53	14.0	80	20.7	60
	Negro	1.6	17	6.4	20	7.2	13	14.4	6
Cerebral arteries	White	0.86	22	1.05	51	2.1	75	3.9	61
	Negro	1.01	16	2.1	19	3.2	13	6.4	6

derived from the clinical histories and autopsy protocols; being based on India standards, the data are not strictly comparable to those of the American group. However, obesity was stated to be present in only 4%, or was only about one-fifth as frequent as in the Los Angeles group.

Effect of Race.—In the Los Angeles group, only the Negroes of 40 years or over provided a sufficient number to invite comparison with Caucasians (Table 3). A lower mean atherosclerotic index was found in both the aorta and the coronary arteries in the Negro, with the exception of the 50- to 59-year age group, in which the indices in the aorta were identical. This result was surprising in view of a greater frequency of stigmata of hypertension in the Negro (39%) than in the Caucasian (30%) in the same decades of life. A slightly but consistently higher atherosclerotic index was found in the cerebral arteries of the Negro as compared with those of the Caucasian.

In considering associated pathologic states which may have a relation to the severity of atherosclerosis, the frequencies of various forms of malignancy were found to be about equal in the two groups, with 26% in Los Angeles and 23% in South India. It might be suggested that the greater frequency of diabetes and hypertension, both of which increase atherosclerosis, might be responsible for the higher atherosclerotic index in the Los Angeles cases. However, the exclusion of both these diseases still resulted in a higher index in the Americans in the after-40 age group. The significance of such factors as heredity,

diet, exercise, and stress cannot be evaluated in this study.

Comment

Atherosclerosis has been evaluated in this study by means of estimates obtained by inspection, a procedure which is justified on the principle that reasonably exact estimates performed without bias on a large number of cases will, when averaged, come remarkably close to the correct value. The separation of extent and severity of atherosclerotic lesions enables one to compare the extent of surface involved by all lesions, the relative proportion of different lesions, and the absolute amount of any single type of lesion. While strictly objective methods, such as mapping the lesions on a transparent film and determining the extent involved by means of a planimeter or by gravimetric methods, would be more accurate, such techniques are extremely tedious and time-consuming. In addition, the presence of minute, scattered, or poorly delineated lesions, which are frequently encountered, would make any purely objective method of evaluation technically difficult.

Our comparison of aortas from patients in the first three decades of life in India with the aortas of a similar group in Los Angeles revealed little difference either in the intimal surface involved or in the severity of lesions. Similar observations have been made in comparing the severity of atherosclerosis of the aorta in New Orleans with that in Guatemala,⁶ and Costa Rica,⁷ and in a study comparing the severity of atherosclerosis in Japan and Guatemala

with that in Los Angeles and New Orleans.⁸ In the present study, it was found that after the third decade, atherosclerosis of both the aorta and the coronary arteries was found to advance more rapidly in the United States than in India.

Information regarding the living habits of the two groups in the present study is extremely limited. While the greater frequency of malnutrition encountered among the South Indians no doubt reflects an impoverished dietary, more comprehensive studies will be necessary before the differences in atherosclerosis can be attributed solely to dietary factors.

Summary

A comparison of atherosclerosis was made in 514 autopsies at the Los Angeles County Hospital and in 259 autopsies in Vellore, South India. In Los Angeles the mean extent of intimal surface involved by atheroma rose most rapidly with increasing age in the aorta, next in the coronary arteries, and least rapidly in the cerebral arteries. An essentially similar relation was found in the involvement of the aorta and coronary arteries in India; cerebral vessels were not studied in this area. In Los Angeles, the extent of atheroma in the female lagged behind the extent in the male in the aorta and coronary arteries during the third, fourth, and fifth decades, but in subsequent decades the severity in the female equaled or exceeded that in the male. The mean extent of intimal atheroma of the coronary arteries was greater in the male in the third through the sixth decades in Los Angeles than in the same sex and decades in India.

During the first three decades, atherosclerosis of the aorta was largely limited to the formation of lipid streaks, and involved approximately the same mean extent of intimal surface in Los Angeles and in South India. In subsequent decades, lipid streaks decreased to an insignificant per cent in Los Angeles, but continued in India at about the same level encountered in the second and third decades. The curve for

plaques was similar in the two geographic areas, except for the steeper rise in the American group from the fourth decade onward. Calcific lesions rose abruptly in Los Angeles in the latter decades of life but remained at a low level in India.

Comparison of the atherosclerotic index of the aorta in the two geographic areas revealed a higher index in Los Angeles after the third decade, a finding which is largely the result of a more rapid increase in plaques and calcific lesions, ulcerative lesions remaining at a low level in the two geographic areas.

Beginning in the fourth decade, the atherosclerotic index was higher in both the aorta and the coronary arteries in Los Angeles than in India, the discrepancy increasing with age.

In Los Angeles, hypertension increased the atherosclerotic index in the aorta in both sexes, but more so in the female, accounting for the higher atherosclerotic index in females in the sixth, seventh, and eighth decades. With the sexes combined, a higher index was found in the coronary and cerebral arteries of hypertensive patients in the sixth, seventh, and eighth decades. The atherosclerotic index was lower in the thin than in the overweight person, but no consistent increase in the index was found in the overweight. The atherosclerotic index was lower in the aorta and coronary arteries and higher in the cerebral arteries of Negroes when compared with Caucasians in Los Angeles.

College of Medical Evangelists.

REFERENCES

1. Snapper, I.: Chinese Lessons to Western Medicine, New York, Interscience Publishers, 1941, pp. 158-161.
2. Kimura, N.: Analysis of 10,000 Postmortem Examinations in Japan, World Trends in Cardiology, edited by A. Keys and P. D. White, Paul B. Hoeber, Inc. (Medical Book Department of Harper & Brothers), 1956, pp. 22-23.
3. Steiner, P.: Necropsies on Okinawans, Arch. Path. 42:359-380, 1946.
4. Higginson, J., and Papler, W. J.: Fat Intake, Serum Cholesterol Concentration, and Athero-

GROSS ESTIMATION OF ATHEROSCLEROSIS

sclerosis in the South African Bantu: II. Atherosclerosis and Coronary Artery Disease, *J. Clin. Invest.* 33:1366-1371, 1954.

5. Gore, I., and Tejada, C.: The Quantitative Appraisal of Atherosclerosis, *Am. J. Path.* 33: 875-885, 1957.

6. Tejada, C., and Gore, I.: Comparison of Atherosclerosis in Guatemala City and New Orleans, *Am. J. Path.* 33:887-894, 1957.

7. Strong, J. P.; McGill, H. C., Jr.; Tejada, C., and Holman, R.: The Natural History of Atherosclerosis: Comparison of the Early Aortic Lesions in New Orleans, Guatemala, and Costa Rica, *Am. J. Path.* 34:731-744, 1958.

8. Gore, I.; Hirst, A. E., Jr., and Koseki, Y.: Comparison of Aortic Atherosclerosis in the United States, Japan, and Guatemala, *Am. J. Clin. Nutrition* 7:50-54, 1959.

Lipidosis of the Liver Portal Spaces

I. A Study of Its Relationship to the Lymphatics of the Liver

G. M. GOLDBERG, M.D., and OTTO SAPHIR, M.D., Chicago

Fatty infiltration and fatty degeneration of the liver are extremely common findings which during the past 20 years have received much attention because of their relation to dietary cirrhosis. Many of these changes are found initially most frequently in the periportal regions. These alterations are quite different from those characterized by the presence of lipids which are confined to the portal areas themselves and which form the basis for this study.

Under normal conditions, fat is histologically visible in only small amounts in the liver, an organ normally concerned in fat metabolism. Disturbances in the amount, distribution, type, or staining quality of various substances in the liver tissues are common in various disease processes.¹ The commonest site of excess accumulation of fat in the liver is in the liver cells, often, at first, only in the periportal areas. The term currently used to denote this entity is "fatty metamorphosis," or "fatty change." Among the many causes are metabolic disturbances, injury to hepatic cells in nutritional deficiencies, anoxia, and certain chemical poisons, such as chloroform, phosphorus, and carbon tetrachloride. There is experimental evidence that the fat which is seen in the hepatic cells is transported metabolic fat derived from food or from fat depots of the body, rather than from unmasking of fat held in the hepatic cells in some other form.²

Although fat accumulates most commonly in the liver cells, it has been noted, both in routine autopsy material and in experimental work,³ that in certain cases lipid vacuoles

appear within the portal areas adjacent to the triads, with or without severe involvement of the liver lobular tissue. Such focal distribution of lipid droplets in the liver portal areas has been described by several investigators,^{3,4} but in general has received only little attention. The origin and mode of transport of these lipid droplets are controversial. Most authors^{1,3} suggested that the fat droplets are carried to the liver by the blood stream. Since the fat in these cases accumulates, at least at first, exclusively in the portal regions away from liver cells, it has nothing in common with fatty degeneration or infiltration. In previous studies it was shown that in follicular lipidosis of the spleen, lipid globules are brought to that organ by a lymphogenous route⁵ and that an intimate relationship exists between the lymphatic system of the spleen and that of the liver.⁶ This relationship was demonstrated by their simultaneous involvement by metastatic carcinoma^{7,8} and by finding of massive accumulations of lymphoid cells within lymphatics in both organs in lymphogenous leukemia.⁹ This communication deals with the significance of portal-space lipid distribution and its relation to the lymphatics of the liver and to those of the spleen.

Material and Methods

This study is based on the examination of necropsy material from 20 cases of follicular lipidosis of the spleen, 10 cases of acute lymphogenous leukemia, 10 cases of chronic lymphogenous leukemia, and 4 cases in which carcinoma was demonstrated to have metastasized to the liver and spleen via the lymphatics. Sections were taken from the liver and spleen shortly after autopsy was performed. The material was fixed in 4% formaldehyde solution U.S.P. and stained with hematoxylin and eosin. Frozen sections were stained with

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From the Department of Pathology, Michael Reese Hospital.

This work was supported by a research grant from Abbott Laboratories, North Chicago, Ill.

LIPIDOSIS OF LIVER PORTAL SPACES

Severity of Lipid Involvement of the Lymphatics* in the Spleen and the Liver

Case No.	Age	Sex	Main Pathological Diagnosis	Degree of Involvement of Lymphatics by Lipids in	
				Spleen	Liver
1	67	M	Adenoca. of colon	+++	++
2	74	F	Ruptured aortic aneurysm	+++	+++
3	61	M	Myocardial infarction	++	++
4	74	F	Bronchopneumonia	+++	+++
5	47	M	Bronchogenic carcinoma	++	++
6	60	M	Bronchiectasis	++	+
7	42	M	Chronic pyelonephritis	++	++
8	56	M	Thrombosis of basilar artery	+	+
9	57	M	Amyotrophic lateral sclerosis	++	++
10	83	F	Infarction of small intestine	++	+
11	76	M	Lipid pneumonia	++++	++++
12	30	M	Astrocytoma of brain	++++	+++
13	64	M	Myocardial infarction	+	+
14	48	M	Spongiblastoma multiforme	++	++
15	58	M	Bronchopneumonia; generalized arteriosclerosis	+	+
16	68	F	Carcinoma of pancreas	++++	+++
17	74	F	Carcinoma of rectum	++	++
18	60	M	Myocardial infarction	++	++
19	60	M	Squamous-cell carcinoma of buccal mucosa	+	+
20	77	M	Bronchiectasis; malignant hepatoma	+++	+++

* The severity of lipid involvement of the lymphatics in the spleen and the liver was graded + to +++++. Case 11, most severely involved, with lipids present in both the liver and the spleen, was graded +++++, while Cases 8, 13, 15, and 19 were least involved and were graded +. All other cases were compared with those two extremes and were accordingly graded.

Sudan IV. The distribution of lipid globules in the portal spaces was compared with that of carcinoma cells^{7,8} propagated via the lymphatics to the liver and to the spleen, as well as with the involvement of the lymphatics of the liver in lymphogenous leukemia.⁶ The material was studied from two aspects: First, an attempt was made to establish whether there was a similar distribution of carcinoma cells, cells of lymphogenous leukemia, and the lipid globules in portal spaces of the liver. Second, the material was studied to see whether the involvement by fat droplets of the spleen and liver represented an involvement of one continuous vascular system. For this purpose 20 cases with follicular lipodosis of the spleen were investigated and served as orientation for the transport of fat. Thus, sections of the spleen were taken to determine the presence and location of lipids. The spleen then served as starting point from where the propagation of the lipids could be followed. To accomplish this, multiple sections were taken in a continuous fashion from the parenchyma of the

spleen, the splenic artery and vein in their extra-splenic route, the celiac trunk region of the splenic and hepatic arteries, the portal vein, hepatic artery, and extrahepatic bile duct, and, finally, from the vessels of the liver hilus region, the perivascular hilar connective tissue, and eventually also from the liver parenchyma. Blocks were cut from all these tissues and fixed in 10% formalin solution. Some of these were embedded in paraffin, cut, and stained with hematoxylin and eosin. From other formalin-fixed blocks, frozen sections were cut, and stained with Sudan IV for the presence of lipids. In all these sections the adventitia of the vessels, together with the loose connective tissue surrounding it, was studied for the presence of lipid globules in lymphatics. It was thought that by a detailed investigation of all these sections it might be possible to demonstrate not only the presence of lipids but also the route used by the fat droplets during their transport to the spleen and the liver or vice versa. It was hoped that the Sudan-positive droplets in all these sections might serve as "landmarks," or "tracers," for the course they followed during their transport. Furthermore, it was thought that this method would also serve to demonstrate whether the lipid was transported by means of blood vessels or lymphatics. As stated, the spleen was used as the starting point, since in a previous study⁶ it was demonstrated that the lipid droplets within the spleen were transported to this organ by perivascular lymphatics. Finally, it was thought that any explanation for the presence of fat in the portal spaces other than their being transported there via the lymphatics could be excluded, provided that no lipid droplets in the hemic vascular bed or other regions of the liver could be demonstrated.

Thus, an effort was made to gather enough evidence for the establishment of one pathogenesis (lymphatic route) for the presence of fat globules in the portal spaces. At the same time it was thought that another pathogenesis (hemic route) would be excluded if no other explanation for the presence of lipid in the portal spaces could be found.

Results

The involvement of the lymphatics in the spleen in three different disease entities has been demonstrated before.⁵⁻⁸ Lymphatics located in the walls of trabecular veins contained carcinoma cells (Fig. 1). Lymphoid leukemia cells involving the lymphatics in the walls of trabecular veins caused the distention and protrusion of these lymphatics into the venous lumen (Fig. 2). Globules (Fig. 3), which on frozen section proved to be

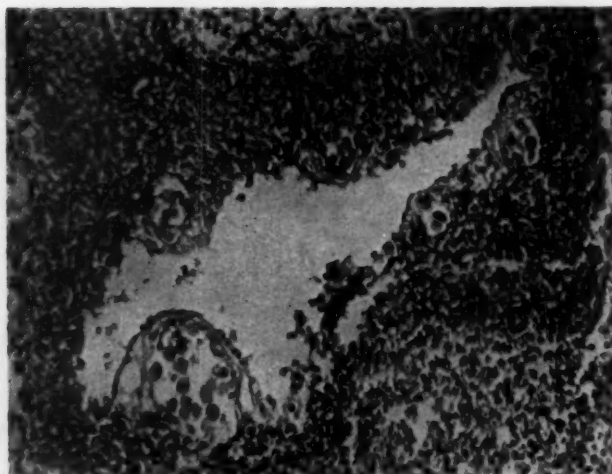


Fig. 1.—Carcinoma propagated via the lymphatics to the spleen. A trabecular vein of the spleen. Note lymphatic vessels with carcinoma cells, bulging into the venous lumen. Endothelial lining of the lymphatics is clearly visible. Van Gieson and Weigert's elastica stain; $\times 300$. (For the use of the histologic section, we are indebted to Dr. H. Ungar, Chairman of the Department of Pathology of the Hebrew University-Hadassah Medical School, Jerusalem).

Fig. 2.—A trabecular vein of the spleen in lymphogenous leukemia. Note lymphatic vessel, with lymphoid leukemia cells, bulging into the venous lumen. Endothelial lining of the lymphatics is not clearly visible because of mesenchymal content and background. Hematoxylin and eosin stain; $\times 300$.

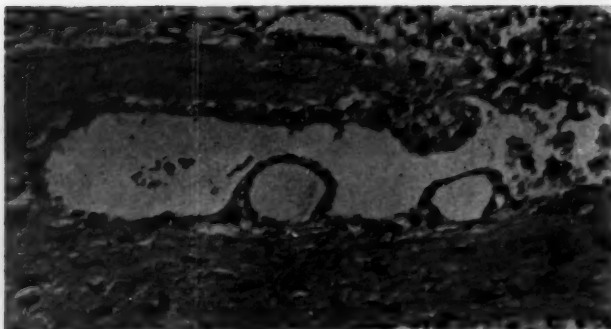
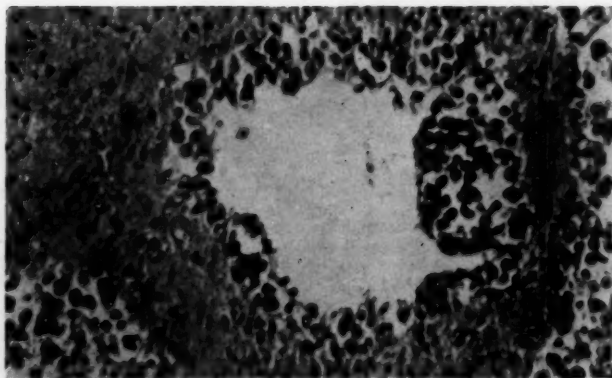


Fig. 3.—Follicular lipoidosis of the spleen. A trabecular vein of the spleen. Note two lymphatic vessels with lipid content (frozen section) bulging into the venous lumen. Endothelial lining of the lymphatics is clearly visible. Compare with Figures 2 and 3. Hematoxylin and eosin stain; $\times 300$.

LIPIDOSIS OF LIVER PORTAL SPACES

Sudan-positive and which filled these lymphatics, brought about their similar bulging into the venous lumen. In this study, involvement of the lymphatics of the liver as well could be easily demonstrated in these three disease entities, and the distribution of the involvement was similar in all three.

When the distribution of lipid globules in the portal spaces of the liver was compared with that of carcinoma metastasizing to this organ via the lymphatics, it was found to be the same in the two entities. Both the carcinoma cells (Fig. 6) and the lipid globules were found in spaces located exclusively in the connective tissue tree accompanying the portal triads (Figs. 4-6). The liver lobules, cords of liver cells, and central vein regions in our cases disclosed neither carcinoma cells nor lipid vacuoles stainable with Sudan IV. A few vacuoles seen at

random within liver cells in the periportal regions were much too scanty in number to allow diagnosing any significant fatty changes. The hemic vascular bed, comprising the sinusoids, portal vein, hepatic artery, and central vein, contained neither carcinoma cells nor lipids.

In a previous work on the localization and significance of leukemic infiltrations in the liver portal spaces in lymphogenous leukemia,⁶ only those cases were chosen in which the portal spaces were exclusively infiltrated with leukemia cells and in which the sinusoids were not involved at all. It was demonstrated in this study that the leukemic infiltrates, in their location, corresponded to the distribution of the lymphatics of the liver, as seen in the portal spaces and capsular regions.

When the distribution of lipid globules (Figs. 4, 5) was compared with that of the leukemic cells within the lymphatics in lymphogenous leukemia, it was again noted that

Fig. 4.—A portal space of the liver in a case with follicular lipodosis of the spleen (Fig. 3). Note the vein filled with red blood cells and, in addition, round, endothelial-lined lymphatics with sudanophilic content on frozen section. Compare with Figures 6 and 7. Hematoxylin and eosin stain; $\times 300$.

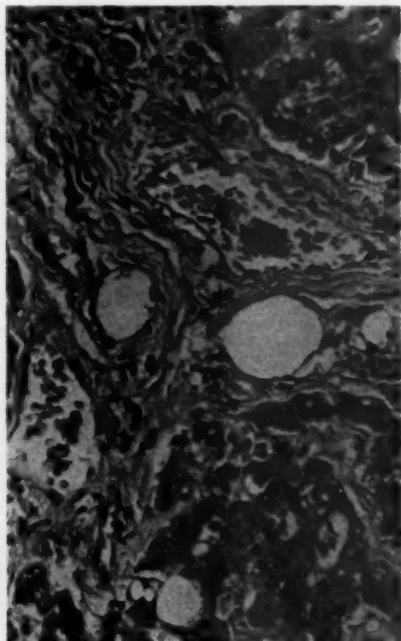
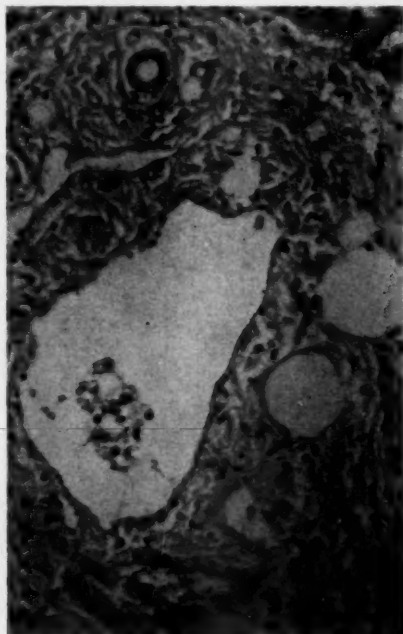


Fig. 5.—Periportal globules (sudanophilic on frozen section) within endothelialized spaces. Note also large vein and bile ducts. Hematoxylin and eosin stain; $\times 300$.



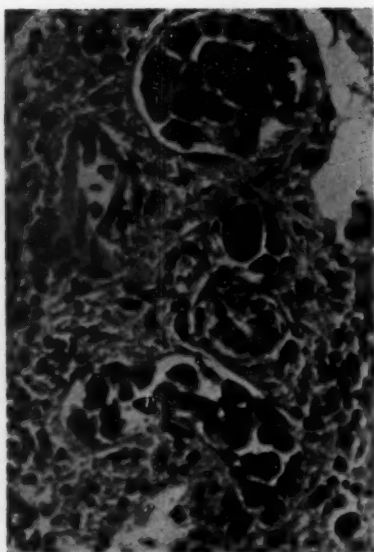


Fig. 6.—A portal space of the liver with carcinoma cells within lymphatics. The bile ducts are visible, as well as the endothelial lining of the lymphatics. Hematoxylin and eosin stain; $\times 300$.

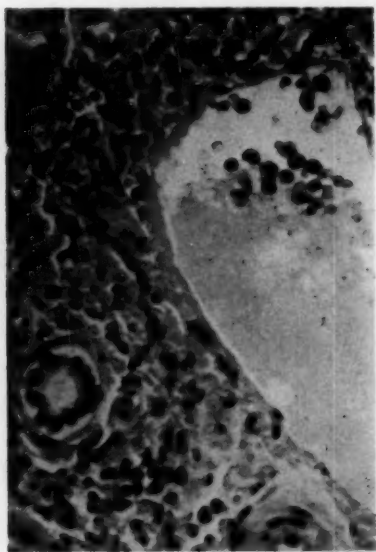


Fig. 7.—A portal space of the liver with accumulation of lymphoid cells in lymphogenous leukemia. Adjacent sinusoids did not show comparable involvement. The infiltrates follow the distribution of the lymphatics, being related to them. Endothelial lining is not clearly visible because of mesenchymal content and background. Hematoxylin and eosin stain; $\times 300$.

the lymphatics of the portal triads (Fig. 7)⁶ were involved, with a striking lack of involvement of other regions of the liver. The capsules of the liver likewise showed parallel involvement of the lymphatics in all three disease entities (Figs. 8-11).

Following the method described before in regard to the route of transport of lipid globules in the spleen, the liver, and the perivascular tissues between these organs, lipid globules were demonstrated within endothelial-lined spaces in the adventitia of the splenic trabecular arteries and veins (Fig. 3). Lipid globules were also found in similar locations in the various sections taken from the hilus of the spleen, splenic artery and vein throughout their course, in the portal ligament, and in sections cut from the hilus of the liver. The lipid accompanied the vessels into the portal areas (Figs. 4, 5) of the liver and remained there. The lipid, in the liver, occupied a perivascular location similar to that observed in the spleen (Figs. 4, 5).

The only vascular systems existing both in the liver and in the spleen are the hemic

and the lymphatic systems. When the hemic vascular bed of the liver and the spleen, comprising the lumens of the arteries, veins, and sinusoids, was investigated for the presence of sudanophilic globules, none could be found there; and certainly no lipid emboli could be demonstrated in either organ within the blood spaces. The lipid vacuoles were exclusively located inside spaces lined by endothelium (Fig. 9). These were located within the portal areas of the liver and its capsule and did not contain any blood corpuscles (Figs. 4, 5). Also, no cellular or nuclear structures could be observed inside the lipid vacuoles. The vacuoles varied in size in the same portal area. They did not form any part of a granuloma of the foreign-body type. They did not elicit any inflammatory reaction and were similar to those observed in the perivascular lipid globules in the spleen. Except in the portal areas, the only other region in the liver where similar sudanophilic vacuoles could be

LIPIDOSIS OF LIVER PORTAL SPACES

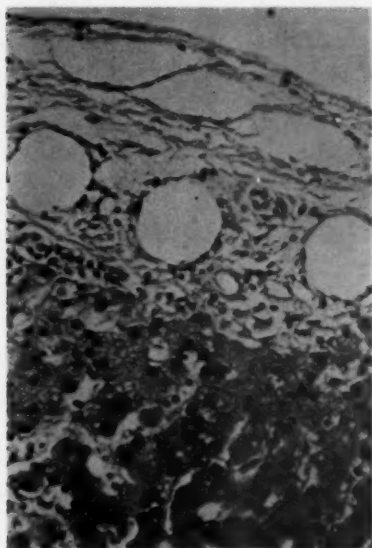


Fig. 8.—Note distended lymphatics with sudanophilic content on frozen section and clearly visible endothelial lining in the capsule of the liver. Compare with Figures 10 and 11. Hematoxylin and eosin stain; $\times 300$.

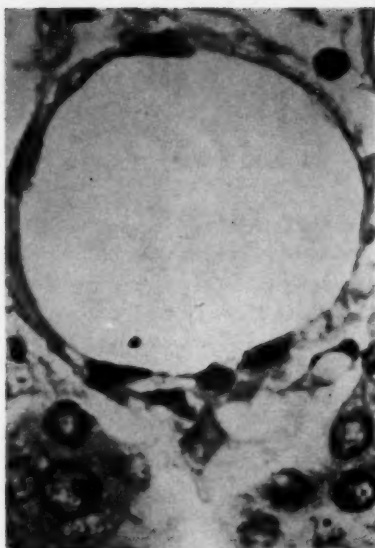


Fig. 9.—A distended lymphatic vessel of the liver capsule. White space in side vessel had a sudanophilic content on frozen section. Note endothelial lining. Hematoxylin and eosin stain; $\times 750$.

Fig. 10.—Carcinoma involving the tissue spaces (lymphatics) of the capsule of the liver. Hematoxylin and eosin stain; $\times 300$.

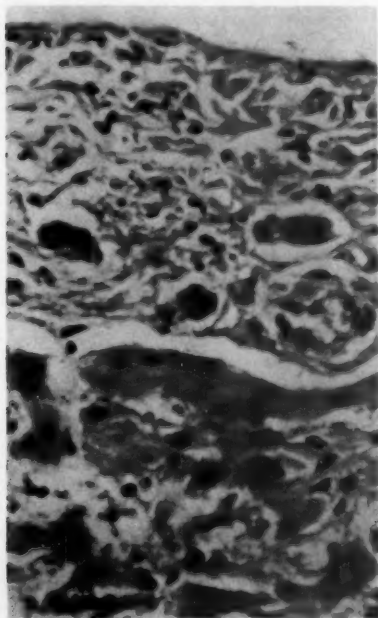
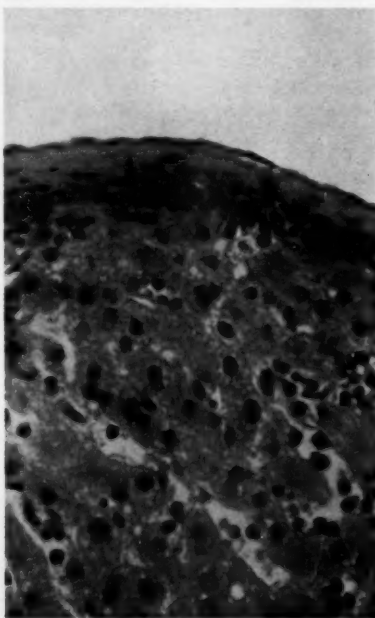


Fig. 11.—Cells of lymphogenous leukemia in the capsule of the liver obviously related to the distribution of the lymphatics. Note severe involvement of capsule as compared with noninvolved adjacent sinusoids. Hematoxylin and eosin stain; $\times 300$.



observed was in the liver capsule (Fig. 8). From the fact that a lining endothelium was often observed surrounding the vacuoles, and that there was never any inflammatory reaction, it seems clear that the spaces are actually lymphatics.

Comment

In evaluating the presence of lipid globules in the liver portal spaces, three methods of comparative pathology were employed. By selecting cases of follicular lipidosis of the spleen in which the fat globules had been demonstrated in the perivascular lymphatics of the spleen, an effort was made to see whether there was a continuous involvement of the lymphatic system with lipid globules, extending from the spleen into the liver. The distribution of the lipid globules in the liver was compared with the distribution of pathologic cells in two other disease entities known to involve the lymphatics of the liver: carcinoma^{7,8} and lymphogenous leukemia.^{6,9} It has been demonstrated previously⁶⁻⁹ that the lymphatic systems of the spleen and the liver are very intimately related and are concomitantly and similarly affected in these two disease entities. Finally, in all the frozen sections taken from the liver, spleen, and the vascular tissues between them, stained with Sudan IV, and carefully examined microscopically, no evidence was found for the presence of fat emboli in the sinusoids, arteries, or veins, either in the spleen or in the liver. This negative finding seems to us to exclude a hematogenous route for the transport of lipid droplets to the liver portal spaces. If the lipid globules were located extravascularly, interstitially in the portal spaces they would behave like any fat found free in tissue spaces under pathologic conditions, and would elicit a foreign-body reaction, or possibly a more acute inflammatory reaction, depending on the nature of the lipid. Such foreign-body granulomas were never observed in the portal spaces. The absence of nuclei in the fat globules and the variability in size of the lipid globules, on the one hand,

and the presence of an endothelial lining around them, on the other hand, exclude their being a part of an adipose tissue. From all the above evidence, it is suggested that the lipid globules present in the portal spaces are located within and transported by the lymphatics. Their relation to periportal and lobular fatty changes of the liver lobules themselves, and their fate, merit further study.

Summary

In 20 cases of follicular lipidosis of the spleen, the liver presented lipid globules in the portal spaces, in the absence of periportal involvement of the liver lobule itself.

Using various modes of approach, especially comparing the localization of carcinoma cells and of lymphoid infiltrates of lymphatic leukemia in the portal spaces with that of the lipid globules, it was found that the latter were located within lymphatics of the portal spaces. No evidence of a hematogenous route in the spread of these lipid globules was found.

It is suggested that lipidosis of the portal spaces may be part of a generalized systemic lymphatic involvement with lipids which reach the liver via the lymphatic route.

The relationship of portal-space lipidosis to periportal and lobular fatty changes of the liver merits further study.

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REFERENCES

1. Anderson, W. A. D., Editor: Pathology, Degenerative Changes and Disturbances of Metabolism, Ed. 3, St. Louis, The C. V. Mosby Company, 1957, pp. 65-66.
2. Jetter, W. W.: Chemical Injury, in Pathology, Ed. 3, edited by W. A. D. Anderson, St. Louis, The C. V. Mosby Company, 1957, p. 149.
3. Stryker, W. A.: Absorption of Liquid Petroleum ("Mineral Oil") from the Intestine: Histologic and Chemical Study, Arch. Path. 31: 670-692, 1941.
4. Pinkerton, H., and Moragues, V.: Paraffinoma of the Lung with Secondary Tubercle-like Lesions in the Liver and Spleen, Arch. Path. 29: 691-699, 1940.

LIPIDOSIS OF LIVER PORTAL SPACES

5. Goldberg, G. M., and Saphir, O.: Follicular Lipidosis of the Spleen: A study of the Mode of Lipid Transport with Reference to the Lymphatics of the Spleen, *Am. J. Path.* 34:1123-1137, 1958.

6. Goldberg, G. M., and Rubenstone, A. I.: A Study of Malignant Lymphomas and Leukemias: I. The Significance of Liver Portal Space "Infiltration" in Lymphogenous Leukemia (with Reference to the Involvement of the Lymphatics), Cancer, to be published.

7. Goldberg, G. M.: Metastatic Carcinoma of the Spleen Resulting from Lymphogenic Spread: Report of 2 Cases, *Lab. Invest.* 6:383-388, 1957.

8. Goldberg, G. M.: The Lymphatics of the Spleen, *J. Anat.* 92:310-314, 1958.

9. Goldberg, G. M., and Rubenstone, A. I.: A Study of Malignant Lymphomas and Leukemias: II. Lymphogenous Leukemia and Myelogenous Leukemia (a Diagnostic Approach with Reference to the Involvement of the Lymphatics of the Liver and the Spleen, Cancer, to be published.

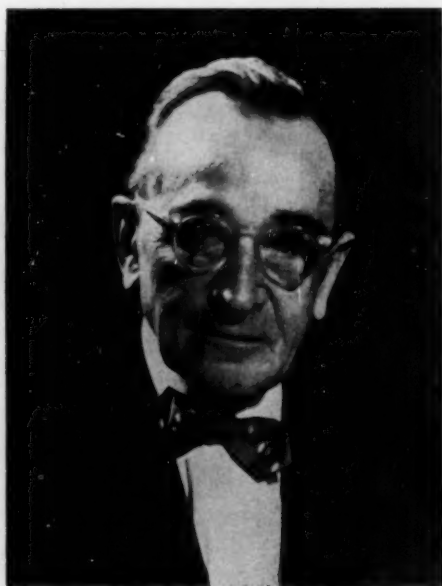
Obituaries

M. C. WINTERNITZ, M.D.

1885-1959

The death of Dr. M. C. Winternitz on Oct. 3, 1959, terminated a long career in pathology. He entered the department at Hopkins in 1907 and retired from the Chairmanship at Yale in 1950. The intervening years were filled with events reflecting his unique character.

His numerous roles in academic and national life attest his many talents. He was Anthony N. Brady Professor of Pathology and Chairman of the department, Dean of the Medical School, Director of the Board of Scientific Advisors of the Jane Coffin Childs Memorial Fund for Medical Research, Chairman of the Committee on the Treatment of War Gas Casualties, Chairman of Division 5 (chemistry) of the Office of Scientific Research and Development, Chairman of the Committee on Growth, Chairman of the Division of Medical Sciences of the



M. C. WINTERNITZ, M.D.

1885-1959

National Research Council, Member of the Hoover Commission. Ideas and the ability and vigor to act potentiated all his various talents and as teacher, dean, investigator, organizer, and administrator, things happened.

He was a dynamic teacher—electrifying is Liebow's word—and it is true that the response to his stimulation could fairly be likened to that from a galvanic current. His purpose was to initiate thought rather than to instruct, to educate in pathology rather than to meet the lesser standards of a qualifying board. As a

OBITUARIES

Dean, he was largely responsible for the Yale Plan, based on the unorthodox assumption that the medical student was an intelligent individual, and his policies and program brought forth the Medical School in its present form. A deep understanding of research, of its methods, and of research men distinguished his conduct of the Childs Fund and the affairs of the National Research Council. The individual rather than the project was significant and worthy of support, and a rare ability to pick the qualified individual made his programs successful. In committee, his clear, concise questions coupled with an immediate plan of procedure abridged controversy, and, although he sometimes persuaded without convincing, his power of expression made him master of every situation.

He had great personal charm, a keen wit, and a high appreciation of wit. He liked good company, chocolates filled with Bourbon whiskey, new ideas, and beagles. He detested obsequiousness, milk, and obituaries.

HARRY S. N. GREENE, M.D.

News and Comment

ANNOUNCEMENTS

Second International Meeting of Forensic Pathology.—The Second International Meeting of Forensic Pathology will be held in New York City on Sept. 18 to 22, 1960. There will be six half-day scientific sessions, to be held at the New York University Post-Graduate Medical School, immediately adjacent to the new office of Chief Medical Examiner. The meeting will have the multiple sponsorship of scientific societies in this country and abroad concerned with forensic pathology and legal medicine. A banquet and other activities are being planned for the entertainment of participants and guests, and their wives and families.

Following the completion of the sessions in New York, those in attendance are invited to Washington to visit the Armed Forces Institute of Pathology, the Federal Bureau of Investigation, the Smithsonian Institute, and other places of interest in the National Capital. The group can then proceed to Chicago for the meetings of the College of American Pathologists and the Society of Clinical Pathologists, which begin on September 25 and extend during most of the following week.

All inquiries may be addressed to:

Dr. Milton Helpert, 55 East End Ave., New York 28

Dr. Francis Camps, 37 Welbeck St., London W.1, England

Dr. Charles Larsen, Tacoma General Hospital, Tacoma, Wash.

Annual Clinical Congress, American College of Surgeons.—The 46th Annual Clinical Congress of the American College of Surgeons will be held on Oct. 10-14, 1960, in San Francisco. For information, write Dr. William E. Adams, Secretary, American College of Surgeons, 40 E. Erie St., Chicago 11.

SOCIETY NEWS

Inter-Society Cytology Council.—The annual scientific meeting of the Inter-Society Cytology Council will be held at the Palmer House, Chicago, on Sept. 23, 24, and 25, 1960. Further information may be obtained from Paul A. Younge, M.D., Secretary-Treasurer, 1101 Beacon St., Brookline 46, Mass.

GENERAL NEWS

Training Program in Experimental Pathology.—The Department of Pathology of the University of Louisville School of Medicine has been awarded a five-year grant of \$152,499 by the U.S. Public Health Service for postdoctoral research training in pathology. The program permits integration of research training with work in anatomical or clinical pathology and with training in other basic medical sciences.

Books

Analytical Cytology.—Edition 2. By Robert C. Mellors, M.D., Ph.D., Editor. Price, \$17.50. Pp. 534, 165 illustrations. The Blakiston Division, McGraw-Hill Book Company, Inc., 330 W. 42d St., New York 36, 1959.

There have been several major revisions in the second edition of this book, which considers physical and chemical methods of analysis of cell structure and function. The section on fluorescence microscopy has been replaced by a chapter written by Robert Mellors dealing exclusively with the "Fluorescent-Antibody Method." The omission of discussion of other types of fluorescence microscopy seems unfortunate, since these are also useful in some situations. Also dropped from this edition are the chapters on ultraviolet microscopy and microspectroscopy, and x-ray diffraction techniques. Titles of the other chapters in this edition are "The Intracellular Localization of Chemical Constituents," by Alex Novikoff; "Phase, Interference, and Polarizing Microscopy," by Robert Barer; "Electron Microscopy," by Cecily Selby; "X-Ray Microscopy," by Arne Engstrom; "Autoradiography in Cytology," by Patrick Fitzgerald, and "The Photometric Chemical Analysis of Cells," by Arthur Pollister and Leonard Ornstein. These chapters all have been revised, and brought up-to-date.

The Clonal Selection Theory of Acquired Immunity. By Sir Macfarlane Burnet. Price, \$5.00. Pp. 209, with 12 figures. Vanderbilt University Press, Vanderbilt University, Nashville 5, Tenn., 1959.

Sir MacFarlane Burnet outlines his current ideas about antibody formation in the Abraham Flexner Lectures for 1958. After a brief discussion of clonal phenomena among bacteria and viruses, Professor Burnet critically evaluates the facts of immunity and emphasizes that all of these must be considered in any theory of antibody formation. He then considers the various theories of antibody production, and he presents his clonal selection theory. This suggests that the capacity to produce specifically patterned proteins, such as antibody, is a genetically determined attribute of cells and that the effect of antigen is simply to stimulate immunologically competent cells to proliferation, antibody production, or other responses. The implication of this hypothesis on immunological tolerance and on various pathological states is then discussed. Finally, he considers clonal selection in neoplastic disease. Much of the material is speculative, but it is presented in a logical and readable manner. Whether or not this theory stands the test of time, it will stimulate new ideas and experimental approaches.

This is a book which must be read not only by immunologists, bacteriologists, and virologists but also by pathologists, hematologists, embryologists, and others interested in the widening aspects of immunology.

The Biochemistry of Clinical Medicine. Second Edition. William S. Hoffman, Ph.D., M.D., F.A.C.P. Price, \$12.00. Pp. 734, with 58 tables and 63 figures. Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1959.

This book is a lively and readable presentation of the important and fundamental biochemical aspects of clinical medicine, designed to help the physician with usual training in biochemistry and physiology to apply modern developments for diagnosis, prognosis, and treatment. Although the content is primarily biochemistry, the treatment and integration of subject matter are at the clinical level and aimed at the clinician rather than the biochemist.

The treatment of subject matter is both extensive and intensive. Chapter headings are rather conventional and include proteins, carbohydrates, lipids, gastric and pancreatic secretions, diabetes mellitus, water and electrolytes, kidneys and urine, nephritis, liver, blood clotting, hemoglobin and the anemias, calcium and phosphorus, thyroid, steroid and other hormones, nucleic acids, vitamins, and biological antagonists. In each case a discussion of fundamental biochemical and physiologic principles precedes the application to clinical problems. Recent developments are well presented and include aminoaciduria, serum proteins and lipoproteins, multiple myeloma, glucagon, oral hypoglycemic agents, metabolism of potassium, bilirubin, porphyrins, biosynthesis of thyroid and adrenal hormones, 17-ketosteroids, plasma and urinary corticosteroids, Wilson's disease, galactosemia, oral diuretics, and serum transaminase.

The organization is logical and readily followed. Interspersed are numerous illustrations from the author's own extensive experience, which add interest and conviction. In complicated or controversial subjects, like liver function tests or factors controlling calcium concentrations of the blood, conflicting views are effectively expressed, followed by the author's logical analysis and reasoned opinion. Relatively few references are given, about 20 to 40 for each chapter, but these are the more valuable for the average reader because they are carefully chosen. The book could well serve as a text for a course, as well as ready reference for the busy clinician. An excellent book, it should be widely used.

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